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(INDIA)

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Impact Damage Characteristics of Soybean

B. D. Bijola, M. S. Gupta, U. S. Agrawal, U. S. Shivhare and B. P. N. Singh
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Impact damage characteristics of soybean were studied at 7.24, 11.60 and 15.10% moisture (d.b.) content under single and double free fall impacting for the drop height range of 0.25 to 5.00 m. Mild steel, cast iron and wood were selected as the impact surfaces. The results indicate that the per cent damage increased linearly with the drop height. The per cent damage was found to increase with number of impacts but decrease with moisture content. Maximum damage occurred when the grains struck the mild steel compared to cast iron and wood surfaces.

Soybean grain is subjected to impact forces of varying magnitudes and nature during post harvest processing. Since the soybean seed coat is fragile, these forces cause grain damage during processing. In certain cases the damage may not be visible, still it may permit direct access to moisture and spoilage agents, such as micro organisms, to the kernel. Therefore, during storage the quality of damaged grains deteriorates at a faster rate than the undamaged ones. As such these impacts are unavoidable but could be restricted to safe limits. This can be done by: (*i*) selecting the appropriate constructional materials, (*ii*) controlling the speed of processing equipments, or (*iii*) modifying the mechanical properties of grains through adjustment of grain moisture.

The rheological properties of soybean were studied by Mohsenin *et al.*[1] They found that the rate of deformation increased the maximum force of ruptures and the force required to initiate the seed coat rupture decreased as the moisture content increased. Bilanski[2] applied compressive loads at the rate of 1.27×10^{-3} min^{-1} to soybean and measured the force and work required to initiate seed coat rupture. He further estimated the energy required for the damage of kernel by giving pendulum impact to the seeds. Fiscus *et al.*[3] found that breakage percentage increased considerably as the drop height increased. The test showed that soybean breakage increased in both cases of grain-concrete and grain-grain impact when dropping height increased. Rajansaroj *et al.*[4] reported that the total damage increased with the increase in drying air temperature, impact velocity and decrease in moisture content of soybean. Maximum rate of toughness was observed at 10 per cent moisture content (dry basis) by Hoki *et al.*[5] Paulson[6] studied the soybean breakage during handling with bucket elevator and screw conveyor. Singh *et al.*[7] reported that the soybean damage decreased with increase in grain moisture content and with decrease in threshing speed. No published information is available on the effect of repeated impacts to the damage of soybean at different grain moistures, impact surfaces and drop height. Therefore, the present work was under taken with the objective of evaluating the damage characteristics of soybean for impact during free fall for different grain moistures, impact surfaces, and drop heights and number of impact combinations.

Materials and Methods

Soybean (variety: 'PK 262') was used for the present study. The harvesting of plants and separation of grains from pods was done manually to avoid any damage during these operations. The moisture content of the grains was brought to 11.6 per cent (d.b.), from the initial 22.6 per cent (d.b.), by sun-drying. The method of free fall of soybean grains from different heights through a vertical pipe to impart the impact was adopted. The schematic diagram of the set-up used for free fall impacting to the grains is shown in Fig 1. The base plate with a central hole, 40 mm diameter, was supported on four levelling bolts. Provision was made to fix the galvonised iron pipes perpendicular to the base plate. A sliding impact plate ($300 \times 50 \times 5$mm size), made up of the material on which the impact was desired, was provided just below the central hole.

The experiments were conducted at twelve different impact heights of 0.25, 0.50, 0.75, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50 and 5.00 m. The impact surfaces were made of mild steel, cast iron and wood and the grain moisture were kept at 7.24, 11.60 and 15.10 per cent (d.b.). Damage characteristics were tested for single and double impact conditions.

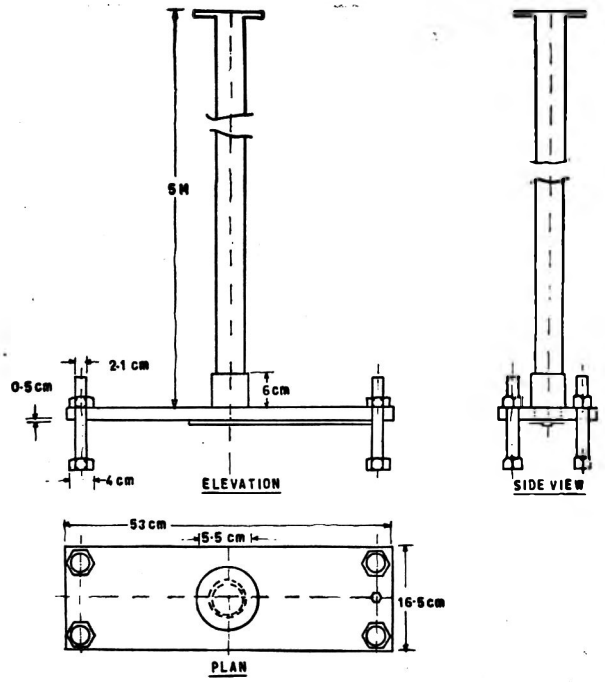

Fig 1. Schematic diagram of experimental set-up.

The soybean grains graded through 5.6 and 4.8mm sieves were used for the study. Impacting was done by dropping the grains one by one from a desired height over the pre-selected impact surface.

The soaking method as described by Young[8] was used to detect the damage of grains. After soaking, the grains were kept in polyethelene bags for 12 hr and then graded. The grains which retained over 5.6 mm sieve were considered to be damaged. The readings were corrected by subtracting the values for control (un-impacted grains). The values of the damage of the control were 8,6 and 6 per cent respectively for the grain moisture content of 7.24, 11.6 and 15.1 per cent (d.b.). For each test a sample of 50 grains was used and the damage expressed in per cent.

Results and Discussion

Since the initial velocity of grain was zero and the force causing the free fall was gravitational force, the impact velocities are functions of drop height only.

For a given combination of number of impacts, drop height and impact surface, the per cent damage was more at lower grain moisture levels. This is in agreement with the findings of earlier workers[3,7]. The per cent damage, in most of the cases was maximum for mild steel, minimum for wood and intermediate for cast iron surface. It was also found that in all the cases the damage varied linearly with the drop height, the relationship being

$$D = k_1 h + k_2 \quad ..(1)$$

where, D is per cent damage; h represents drop height in meter and k_1 and k_2 are regression constants.

The calculated values of the constants k_1 and k_2 and coefficient of correlation, r, are given in Table 1. Sample data plots in terms of the variables of equation 1 are shown in Fig 2 and 3. Values of constants k_1 and k_2 decreased with the increase in grain moisture content and increased with the increase in number of impacts; and were maximum for mild steel, minimum for wood and intermediate for cast iron surface. This indicates that the effects of drop height and number of impacts are additive in nature but opposite to the effect of grain moisture content for a given impact surface. The data were analysed statistically which confirms the inferences drawn and the significance of various treatments at one per cent and their two factor interactions at 5 per cent level of significance.

Table 1. Values of the constants k_1 and k_2 and the coefficient of correlation

No. of impacts	Grain moisture (%)	Constants k_1	Constants k_2	Coeff. correlation (r)
		Wood		
Single	7.24	6.703	0.072	0.984
	11.60	5.514	-0.550	0.990
	15.10	4.597	-1.924	0.985
Double	7.24	7.457	1.650	0.977
	11.60	6.311	0.932	0.979
	15.10	5.148	-0.517	0.988
		Cast iron		
Single	7.24	6.803	3.084	0.973
	11.60	7.752	-1.458	0.987
	15.10	5.641	1.010	0.988
Double	7.24	8.458	4.687	0.982
	11.60	7.256	4.861	0.972
	15.10	5.880	2.474	0.982
		Mild steel		
Single	7.24	8.428	3.368	0.983
	11.60	7.248	3.185	0.987
	15.10	5.988	1.487	0.986
Double	7.24	8.788	6.425	0.972
	11.60	8.185	5.439	0.982
	15.10	6.053	5.652	0.969

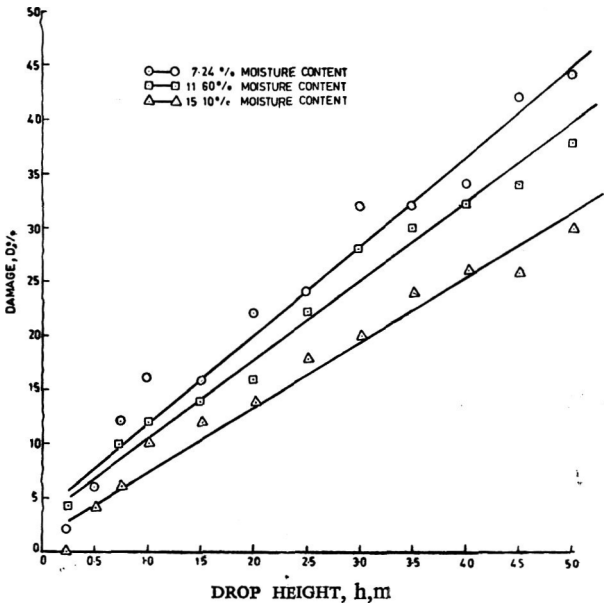

Fig 2. Effect of drop height on damage at different moisture levels for single impact on mild steel surface.

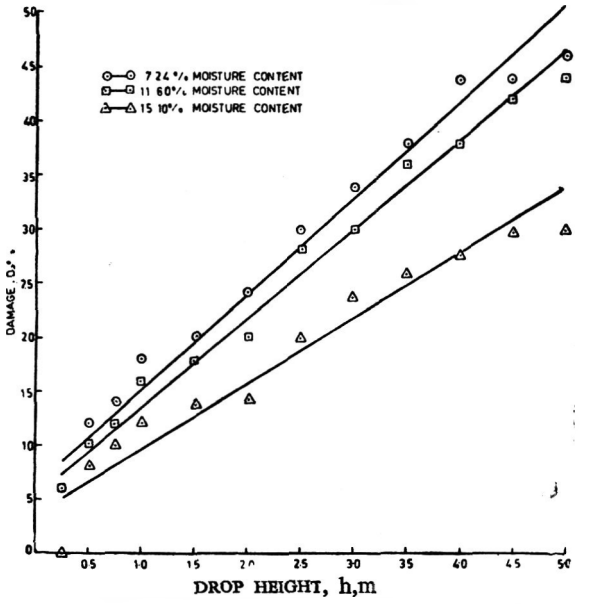

Fig 3. Effect of drop height on damage at different moisture levels for double impact on mild steel surface.

The reason for this behaviour of soybean grains may be that the damage caused is a function of the energy dissipation and its rate. The kinetic energy, in present case is proportional to the drop height and hence the damage increases linearly with the increase in drop height. Theoretically this trend is possible only upto a certain value of drop height beyond which the damage would start increasing at a decreasing rate. Under the present experimental conditions the change was linear throughout the range for most of the treatments.

For a given value of the kinetic energy (i.e. height) the rate of loading would depend upon the modulii of elasticity of colliding materials namely impact surface and grain. For a higher value of these modulii the rate of loading would be higher. Since the modulus of elasticity of grains is envisaged to be higher at lower moisture contents, the rate of loading would also be high. This explains the effect of grain moisture content on damage. The values of modulii of elasticity of wood, cast iron and mild steel are $9\times10^4 - 13\times10^4$, $98\times10^4 - 162\times10^4$ and $204\times10^4 - 218\times10^4$ kg/cm^2 respectively i.e. in increasing order[10]. This must have resulted in maximum grain damage in mild steel and minimum for wood surface.

The damage caused by double impact was found to be more than that by single impact. The reason for this may be that the some of the grains which have been identified as undamaged during the single impact, might have suffered minor injuries, and have become more susceptible to further damage. Such grains, when subjected to subsequent impact, must have got damaged resulting in higher per cent damage in the case of double impact.

From these observations it could be concluded that damage: (*i*) increases linearly with the drop height, (*ii*) increases with the number of impacts (*iii*) decreases as the grain moisture increases and (*iv*) is greater for mild steel surface than wood and cast iron.

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Optimization of Operating Parameters of a Centrifugal Sheller for Dehusking 'Jaya' Variety Paddy

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Experiments were conducted on raw and parboiled paddy, variety 'Jaya' to optimize the operating parameters of a centrifugal sheller for maximum total and head yield per unit of energy consumption. The parameters considered were feed rate and impeller speed. The feed rate was varied between 150 and 270 kg/hr and the impeller speed was varied between 2250 and 3300 rpm. The overall performance of the sheller was expressed by performance index. The sheller gave optimum rice out turn at an impeller speed of 2700 rpm for raw paddy at 13.5% moisture content (w.b.) and 2650 rpm for parboiled paddy at 14.25% moisture content (w.b.) at an optimum feed rate of about 240 kg/hr.

It has been estimated that about 10 per cent of paddy produced is lost in various traditional harvest and post harvest operations[1], such as threshing, transporting, handling, drying, milling and storage. By the use of efficient post harvest practices, the losses can be minimised. Among the rice processing operations, shelling is the single important operation which calls for a major effort in order to achieve a quantitative increase and qualitative improvement in rice production in the country.

In India, various machines are used for shelling paddy. Centrifugal sheller is considered as a low capacity, modern paddy shelling machine by the Government of India. Khadi and Village Industries Corporation has considered it as the best paddy shelling machine for rural areas and has recommended its use in place of hullers. Centrifugal shellers are being manufactured by a few well known firms. The list of manufacturers with capacity and power requirement of the machine has been given in Appendix I.

Materials and Methods

Centrifugal sheller has only one moving part that is the impeller. The impeller casing is lined with hard rubber. The grains which are fed in the centre are guided through the impeller blades by centrifugal force. After leaving the impeller, they strike the rubber lining at a certain angle and slide with it. Due to impact, husk gets opened and due to sliding on the stationary hard rubber surface, shelling takes place[2].

In a sheller there are two parameters i.e. feed rate and impeller speed which can be varied so as to optimize the output. The other parameters like diameter of the impeller, number of blades, inlet blade angle, outlet blade angle, etc are constant for a particular sheller. The sheller currently used has an impeller diameter of 220 mm; three blades with an inlet angle of 60° and outlet angle of 75°. The feed rate of paddy and impeller speed could be varied. The manufacturer's recommended feed rate is 200 kg/hr and impeller speed is 3700 rpm. Therefore the feed rate was varied between 150 and 270 kg/hr and imepller speed was varied between 2250 and 3300 rpm. Preliminary investigations indicated a very high per cent brokens (40.56 and 19.16 for raw and parboiled paddy, respectively) at 3700 rpm. In the laboratory rubber roller sheller, the per cent brokens were 22.56 and 0.65 for raw and parboiled paddy, respectively (Table 1). The maximum imepller speeds of 3000 rpm and 3300 rpm were selected for shelling raw paddy and parboiled paddy respectively so as to obtain optimum quantity of head rice. At the above selected maximum impeller speeds, for different feed rates, the per cent

TABLE 1. SHELLING EFFICIENCY AND PERCENT BROKENS AS A FUNCTION OF METHOD OF SHELLING AND TYPE OF EQUIPMENT USED IN DEHUSKING PADDY

Shelling method/ equipment	Shelling efficiency (%)		Brokens %	
	Raw paddy	Parboiled paddy	Raw paddy	Parboiled paddy
Hand shelling	100.00	100.00	20.76	0.00
Lab. rubber roll sheller	99.03	99.48	22.56	0.65
Centrifugal sheller				
A. At manufacturer's recommendation	96.20	97.56	40.56	19.16
B. At optimum condition	66.69	77.31	18.10	1.42

Moisture content: raw paddy 13.50% (w.b.), parboiled paddy 14.25% (w.b.),

Manufacturer's recommendation for centrifugal sheller: feed rate 200 kg/hr; impeller speed 3700 rpm.

Optimum condition for centrifugal sheller for 'Jaya' variety paddy: Feed rate 240 kg/hr for raw and parboiled paddy. Impeller speed 2700 rpm for raw paddy and 2650 rpm for parboiled paddy.

brokens varied from 24.27 to 32.51 for raw paddy and from 5.25 to 5.33 for parboiled paddy during the shelling.

Freshly harvested paddy, variety 'Jaya' was procured from the farm. After cleaning, half the quantity was parboiled following CFTRI[3] method. Raw and parboiled paddy were dried in the sun to a final moisture content of around 14 per cent on w.b. In actual, the moisture content (w.b.) varied between 13.5 and 14.5 per cent. The experimental set up consists of centrifugal sheller assembly and its supporting structure, main shaft and its stand, power supply and power transmission units. The power transmitted to the sheller through V-belt and pulley arrangement from a 2 hp DC motor which is equipped with a variable speed rectifier-cum-controller. By monitoring the power supply, the impeller speed was varied to get any required speed. The feed rate was varied by adjusting the inlet opening.

The feed rate was determined by collecting the total output material from the sheller for a predecided time. During the experiments, three samples were taken randomly for visual analysis. All the data reported are the average values of three observations. Power consumption was recorded using a voltmeter and ammeter. The shelling efficiency, sheller index and performance index were calculated by using the equation developed by Jain *et al.*[4]

$$S = \frac{X_w + X_b}{X_t} \quad ..(1)$$

$$SI = \frac{X_w}{X_h \cdot X_t} \quad ..(2)$$

$$PI = \frac{X_w}{X_h \cdot X_t \cdot E_s} \quad ..(3)$$

Where

- S .. Shelling efficiency in per cent
- SI .. Sheller index in fraction
- PI .. Performance index in T/KWH
- X_w .. Ratio of the weight of whole brown rice in the out product to the total weight of the paddy grains fed.
- X_b .. Ratio of weight of broken brown rice in the out product and the total weight of paddy grains fed.
- X_t .. Actual fraction of brown rice (whole+ brokens) in the feed.
- X_h .. Actual fraction of the whole brown rice in the feed.
- E_s .. Specific energy consumption in KWH/T paddy shelled.

Results and Discussion

Shelling efficiency, sheller index and performance index were calculated and are shown in Fig 1 - 6 as a function of impeller speed at different feed rates.

Effect of impeller speed on the performance of centifugal sheller: As the speed increased, the shelling efficiency

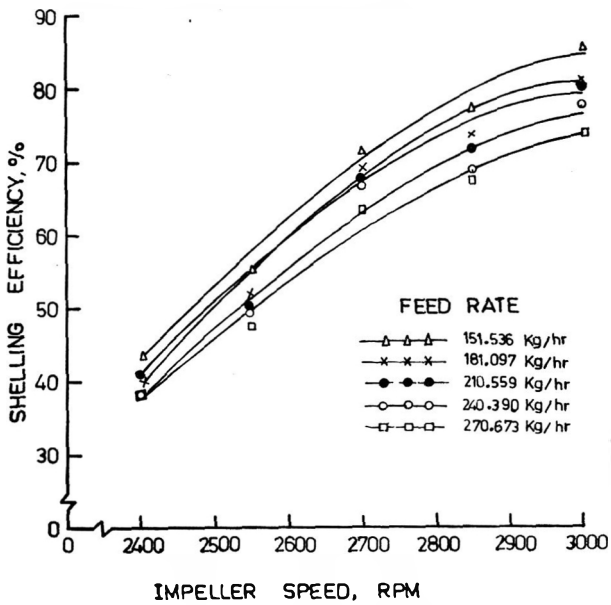

Fig 1. Impeller speed Vs shelling efficiency for raw paddy, variety 'Jaya' at 13.50% moisture content (w.b.)

increased at a decreasing rate for raw paddy at all the feed rates studied (Fig 1). When the speed was varied from 2400 to 3000 rpm, the lowest efficiency of 37.95 per cent was observed at a feed rate of 270 kg/hr and the maximum efficiency of 85.58 per cent was observed at a feed rate of 150 kg/hr.

Similar trend of increase of efficiency with the increase of impeller speed was observed for parboiled paddy (Fig 2). However, in this case the shelling efficiency inceased at an increasing rate as the speed is increased from 2250 to 3300 rpm. The minimum efficiency observed was 55.70 per cent at the feed rate of 260 kg/hr and the maximum efficiency observed was 97.38 per cent at a feed rate of 170 kg/hr. Better shelling efficiency was found at higher impeller speeds. When grain starts rotating along with the impeller at its inlet, it is subjected to the centrifugal force. Also the grain which moves from inlet to outlet has to face friction between grain to grain and impeller surfaces. The centrifugal force which is exerted on the particle at the time it leaves the periphery is a function of impeller speed. Higher impeller speeds induce larger impact and shear forces which in turn improves shelling efficiency.

The effect of impeller speed on sheller index at different feed rates is given in Fig 3 for raw paddy and in Fig 4 for parboiled paddy. In both the cases, the sheller index

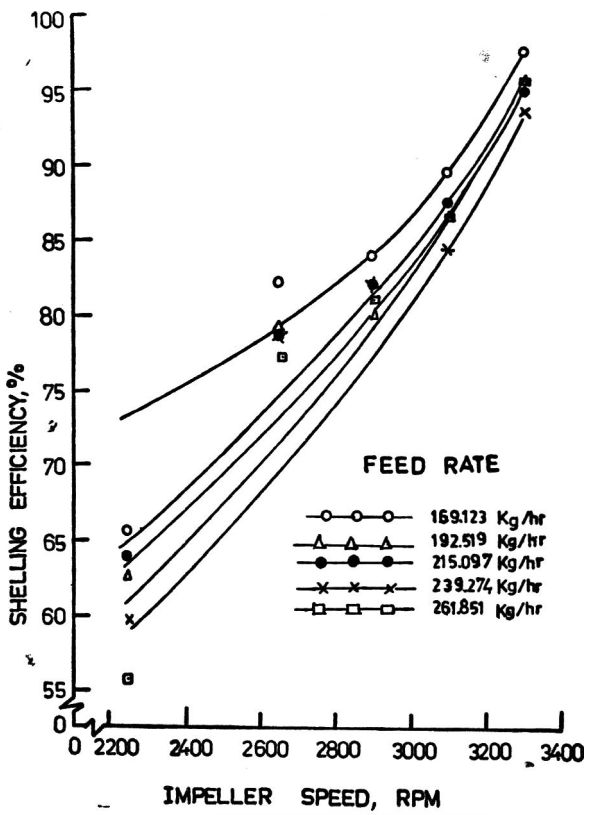

Fig 2. Impeller speed Vs shelling efficiency for parboiled paddy, variety 'Jaya' at 14.25% moisture content (w.b.)

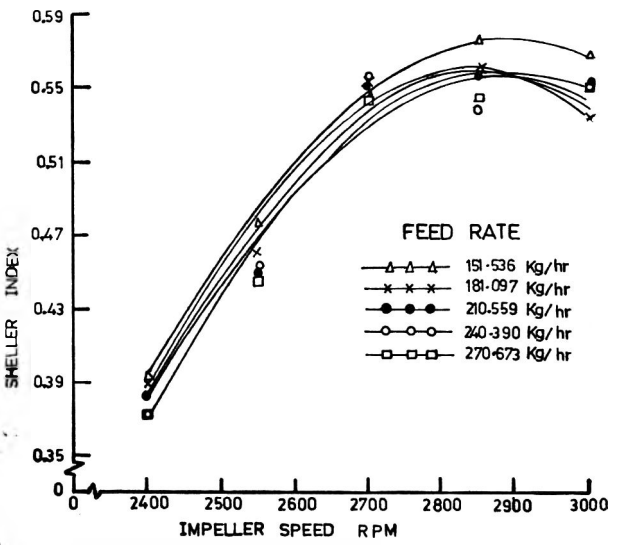

Fig 3. Impeller speed Vs sheller index for raw paddy, variety 'Jaya' at 13.50% moisture content (w.k.)

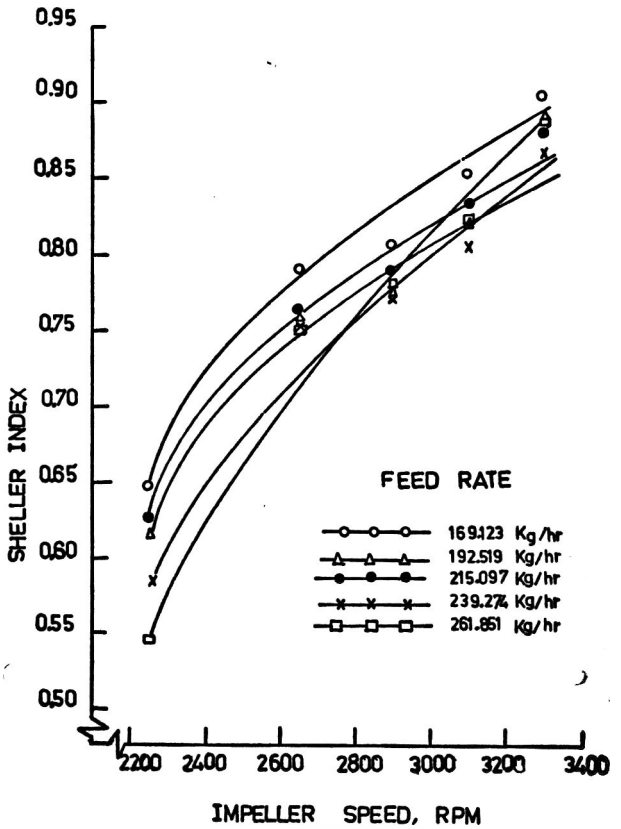

Fig 4. Impeller speed Vs sheller index for parboiled paddy, variety 'Jaya' at 14.25% moisture content (w.b.)

is increasing with the increase of impeller speed. However, for raw paddy a peak point was observed at about 2850 rpm, beyond which the sheller index decreased. Per cent brokens were taken into account in the

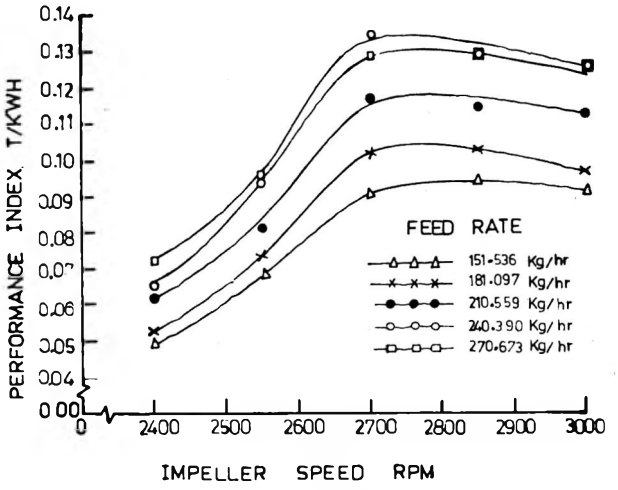

Fig 5. Impeller speed Vs performance index for raw paddy, variety 'Jaya' at 13.50% moisture content (w.b.)

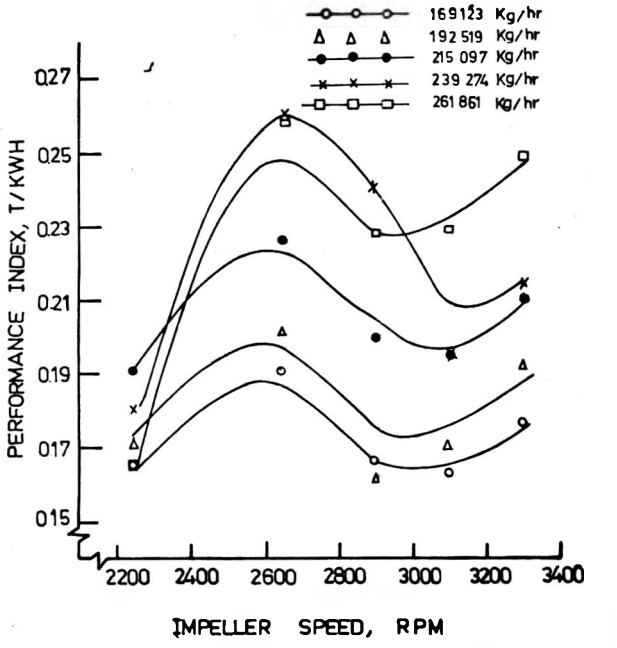

Fig 6. Impeller speed Vs performance index for parboiled paddy, variety 'Jaya' at 14.25% moisture content (w.b.)

derivation of equation for sheller index[4]. Per cent brokens increased with the increase of impeller speed at all feed rates. Below critical speed, the shelling efficiency increased at a faster rate compared to increase of per cent brokens. However, at higher impeller speeds due to larger impact and shear forces, there was significant increase in per cent brokens which in turn decreased per cent head yield of rice significantly. On the other hand, in case of parboiled paddy, per cent brokens varied between 1.00 and 5.56 per cent, whereas shelling efficiency varied between 55.70 and 97.38 per cent. This shows that there was no significant decrease in per cent head yield of rice which in turn indicates that parboiled grains can take up larger impact and shear forces compared to raw paddy grains.

The relationship between performance index and impeller speed for different feed rates is shown in Fig 5 for raw paddy and in Fig 6 for parboiled paddy. The performance index increased with the increase in impeller speed till a peak point is reached; beyond that point, the performance index decreased with the increase in speed. As the performance index is the ratio of sheller index to specific power consumption, the behaviour observed is anticipated. Both sheller index and specific power consumption increased with the increase of impeller speed, but at different rates. At initial stages, the sheller index increased at a faster rate compared to the increase of specific power consumption. Therefore, the performance index increased.

As the speed is increased beyond the critical value, the sheller index decreased with the increase of impeller speed. Therefore, the performance index decreased significantly with the increase in impeller speed. However the decrease in performance index started below critical speed. It seems that there is a point beyond which the rate of increase of sheller index is decreasing with the increase in impeller speed. On the other hand, specific power consumption may be maintaining the same rate of increase. Therefore, maximum performance index was observed at 2700 rpm for raw paddy and 2650 rpm for parboiled paddy.

Effect of feed rate on the performance of centrifugal sheller: For both raw and parboiled paddy, shelling efficiency decreased with the increase in feed rate at all impeller speeds. As the feed rate increased, number of grains entering the sheller per unit time are also increasing. It is possible that at low feed rates, all the grains are hitting the hard rubber surface and sliding along with it. However, when the feed rate is increased, some grains may be hitting the paddy grains instead of hitting the hard rubber surface and they may be sliding on the grains. The impact and shear forces in these cases may not be sufficient for dehulling raw paddy. As the feed rate is further increased, the number of grains hitting grain surface may also be increasing resulting in further decrease of shelling efficiency. When the feed rate is increased from 150 to 270 kg/hr, the decrease in shelling efficiency varied from 10.88 to 14.27 per cent compared to shelling efficiencies at 150 kg/hr. On an average the shelling efficiency decreased by about 12.31 per cent in raw paddy and 3.72 per cent in parboiled paddy except for the impeller speed of 2250 rpm. In the case of parboiled paddy, the force required to dehull paddy is much less compared to raw paddy as the husk gets split during parboiling[5]. Therefore, though the grains may be hitting and sliding on the grain surface, the decrease in

shelling efficiency is insignificant in the case of parboiled paddy.

The effect of feed rate on sheller index is insignificant. Keeping impeller speed constant, when feed rate is increased, both shelling efficiency and per cent brokens decreased. As a result, sheller index remained almost constant.

Performance index of the sheller increased with the increase of feed rate. This may be due to decrease in specific energy consumption with the increase of feed rate at a constant impeller speed. In absolute terms there is no significant increase in power consumption with the increase in feed rate. As the specific energy consumption is the ratio of power consumption and paddy shelled, it decreases with the increase in feed rate.

Performance index indicates the overall performance of the sheller. Therefore, the values of operating parameters at the peak value of performance index indicates the optimum operating conditions. Maximum performance index of 0.1345 T/KWH was obtainedfor raw paddy at a feed rate of 240 kg/hr and an impeller speed of 2700 rpm. Similarly maximum performance index of 0.2612 T/KWH was obtained for parboiled paddy at a feed rate of 240 kg/hr and an impeller speed of 2650 rpm. These are the optimum operating conditions for the centrifugal sheller tested. Similar tests can be conducted on different makes of centrifugal shellers to optimize the operating parameters.

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APPENDIX—I

FIRMS MANUFACTURING CENTRIFUGAL SHELLER AND THEIR SPECIFICATIONS

Sl. no.	Name and address of the manufacturer	Particulars	Capacity (kg/hr)	Power requirement (hp)	Price Rs.
1.	M/s. A.K.J. Engineering Works, Gundusalai Road, Alpettai Manjakuppam, Cuddalore-607 001, Tamil Nadu.	Centrifugal dehusker	2000 – 3000	5	19,000
2.	M/s. Dev Raj and Co., Krishna Sudama Marg, Firozpur City-152 002.	Centrifugal dehusker	300 – 400	3	6,100
3.	M/s. Kisan Krishi Yantra Udyog, 64, Moti Bhawan, Collectorganj, Kanpur-208 001.	(*i*) Dehusker-cum-separator (*ii*) Composite unit	400–500 200	3 5	15,500 14,500
4.	M/s. K.L.N. Krishnan and Sons, Engineering Division, 69, South Veli Street, Madurai-625 001.	Single centrifugal dehusker	400 – 500	1	3,250
5.	M/s. Mysore Industries, No. 2336, 9th Cross, Basaveswara Road, Mysore-570 004.	Modern paddy dehusker C.S. type (*a*) without husk fan (*b*) with husk fan	300 – 500	 2 2.5	 4,500 5,500
6.	M/s. Mysore Precision Engineers, C-123, Industrial Estate, Yadavagiri Mysore-570 020.	Composite unit includes paddy cleaner, C. sheller, husk aspirator, rice polisher.	500	9	42,500
7.	M/s. Navin Industries, 180, Linghi Street, Madras-600 001.	Centrifugal paddy dehusker	400	2	5,500

Source: *Directory-Rice Milling and Allied Machinery in India*, Regional Extension Service Centre, PHTC, I.I.T., Kharagpur, 1984.

Effect of Enzyme Active Soy Flour and Roasted Soy Flour on Rheological and Baking Characteristics of Wheat Flour

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Wheat-soy flour blends in the ratio of 100:0, 97:3, 94:6, 91:9, 88:12 85:15, and 82:18 were prepared using enzyme active soy flour and sand roasted (200±4°C) soy flour. The addition of either type of soy flour increased the mixing tolerance, mixing time, dough stability, elasticity and deformation work and decreased developing area, extensibility and baking strength. However, the effects were more pronounced with enzyme active soy flour as compared to roasted soy flour. On the ether hand, loaf volume, loaf height, texture, crumb colour and grain, crust colour and flavour of bread were affected to lesser extent with roasted soy flour as compared to enzyme active soy flour. Organoleptic studies revealed that the addition of roasted soy flour upto 12% gave a satisfactory bread which was almost equal to that produced by 9% incorporation of enzyme active soy flour. Roasted soy flour can be used in place of enzyme active flour for fortification of wheat flour in bread making.

The problem of protein energy malnutrition (PEM) has attracted the attention of many scientists towards the fortification of bread or other cereal products with protein rich materials[1] like soybean flour. However, supplementation of wheat flour with soy flour reduces its bread making potential and consequently requires technological measures and/or the use of dough strengtheners to produce bread which is acceptable[2]. So far the enzyme active soy flour, soybean flour prepared by conventional method and defatted soy flour with improvers have been tried to fortify wheat flour for making bread.[3-5] The present work was undertaken to make a comparative study of the effect of roasted as well as enzyme active full fat soy flour on the baking quality of wheat flour.

Materials and Methods

Soybean ('Bragg' variety) and wheat ('U.P. 262') were obtained from G.B. Pant University of Agriculture and Technology, Pantnagar. After cleaning manually to remove extraneous matter the wheat was conditioned at 15 per cent moisture content for 48 hr and milled in a laboratory Buhler mill (Model MLU 202). A portion of soybean sample was dehulled and the cotyledons were milled using a Pin Mill to obtain enzyme active soy flour. The remaining portion was roasted along with sand at 200±4°C for 8 sec. Roasted soybeans were dehulled by hand pounding and the hulls were separated by sieving. The roasted cotyledons were also milled using Pin Mill to get roasted soy flour. Blends of wheat with enzyme active soy flour as well as roasted soy flour were prepared in the ratios of 100:0, 97:3, 94:6, 91:9, 88:12, 85:15 and 82:18. The blends were mixed and sifted through a 60 mesh sieve for uniform mixing.

Chemical analysis: Wheat flour, soy flour and their blends were analysed for proximate composition using standard AACC methods[6].

Physical dough tests: Mixograph and Chopin Alveograph were used to study the dough characteristics of wheat flour and wheat-soy flour blends.

Test baking: Straight dough method[6] was used for test baking. Bread from each blend was made by taking 100 g flour; 2.5 g yeast; 1.0g, salt; 2.5g, sugar, 2.0g, shortening and water as required to bring the dough to desired consistency under the following conditions: mixing time 2.0 min (Hobart mixer); bulk fermentation 105 min at 30°C and 75 per cent R.H.; remixing 2 min; recovery time 50 min; remixing 2 min and refermentation 25 min; proofing 55 min and baking at 435°F for 22 min.

Bread characteristics: The bread characteristics measured included loaf volume, loaf weight, loaf height and specific loaf volume. Rapeseed displacement method was used to measure loaf volume.

Sensory analysis: The cooled loaves were stored in closed tin containers overnight and were evaluated for crust, crumb and eating qualities using a panel of 10 members selected from the Department of Food Science and Technology. The panelists were asked to evaluate

the breads using a total score of 100 comprising general appearance, 20; crust colour, 10; grain structure, 20; texture, 25 and taste and odour, 25.

All the analyses were done in duplicate and the average values have been reported.

Results and Discussion

The protein content of wheat flour, enzyme active soy flour and roasted soy flour was 12.6, 44.3 and 39.3 per cent and the ash content was 0.6, 4.9 and 5.2 per cent dry basis, respectively.

Mixographic characteristics: The results showing the effect of enzyme active soy flour as well as roasted soy flour on the mixograph characteristics of wheat flour are presented in Table 1. The developing angle and weakening angle which reflect the rate and manner of dough development decreased whereas the mixing tolerance and mixing time increased with increasing proportion of soy flour. However, the rate of decrease in both the angles was more rapid in enzyme active soy flour blends as compared to roasted soy flour blends and hence it exhibited greater effect on the mixing tolerance of dough. This might be attributed to the activity of lipoxygenase present in enzyme active soy flour. Hoseney *et al.*[7], Faubion and Hoseney[8] and Keiffer and Grosch[9] reported similar effect of soy flour addition on the mixing tolerance of wheat flour.

The mixing time of the blend of wheat flour and enzyme active soy flour ranged from 3.1 to 5.8 min at 0 and 15 per cent level of enzyme active soy flour, respectively. On the contrary the mixing time of wheat flour and roasted soy flour blend ranged from 3.1 to 4.1 min respectively.

The peak width and peak height of the mixogram patterns of wheat flour and soy flour blends decreased with increasing level of soy flour. However, the decrease was more perceptible with enzyme active soy flour than with roasted soy flour. This might be due to the greater water absorption of enzyme active soy flour. Baig and Hoseney[10] reported that the peak height decreased with the increase in water absorption.

Alveographic characteristics: The alveographic characteristics of wheat flour as affected by the addition of soy flour are presented in Table 2. Dough stability, baking strength, elasticity and deformation work increased with the increase in level of soy flour whereas the extensibility, the ratio of extensibility to stability (L/P ratio) and swelling index decreased. However, the increase in dough stability and elasticity was more pronounced with enzyme active soy flour. On the contrary, the increase in baking strength was much greater with roasted soy flour compared to enzyme active soy flour. Similar effect of soy flour addition on baking strength and elasticity was observed by Austin[11] and Rao and Vakil[12]. The increase in deformation work, which reflects resistance to extension was more conspicuous with 9 per cent enzyme active soy flour than 12 per cent roasted soy flour. The enzyme active soy flour exhibited greater effect on dough extensibility, L/P ratio and swelling index as compared to roasted soy flour.

Bread characteristics: Addition of enzyme active soy flour showed consistently decreasing effect on the loaf height and loaf volume of bread whereas the addition of roasted soy flour at 9 and 6 per cent level increased the loaf height and loaf volume, respectively. (Table 3). The loaf weight of breads made from wheat flour-soy flour blends increased from 136.5 to 142.8g with roasted soy

TABLE 1. EFFECT OF BLENDING ENZYME ACTIVE SOY FLOUR AND ROASTED SOY FLOUR ON MIXOGRAPHIC CHARACTERISTICS OF WHEAT FLOUR

Wheat flour in blend (%)	Developing angle X°		Weakening angle Y°		Mixing tolerance 180°-(X+Y)°		Mixing time (min)		Peak width (cm)		Peak height (cm)		Developing area (cm^2)	
	WE	WR	WE	WR	WE	WR	WE	WR	WE	WR	WE	WR	WE	WR
100	27	27	14	14	139	139	3.1	3.1	2.1	2.1	6.0	6.0	20.4	20.4
97	25	25	15	14	140	141	3.9	3.8	1.7	1.7	5.8	5.9	29.7	23.9
94	20	20	3	10	157	150	4.7	4.3	1.1	1.4	5.3	5.5	26.6	27.1
91	15	17	2	7	163	156	5.2	4.0	1.1	1.3	4.5	5.2	25.8	24.3
88	10	16	0	3	170	161	5.6	4.5	1.0	1.2	4.1	4.8	24.7	22.9
85	11	16	0	4	169	160	5.8	4.7	0.9	1.2	4.0	5.1	25.5	26.8
82	—	9	—	3	—	168	—	5.4	—	1.1	—	4.6	—	30.6

WE=Wheat flour and enzyme active soy flour blend

WR=Wheat flour and roasted soy flour blend

TABLE 2. EFFECT OF BLENDING ENZYME ACTIVE SOY FLOUR AND ROASTED SOY FLOUR ON ALVEOGRAMS OF WHEAT FLOUR

Wheat flour in blend (%)	Stability, P (mm)		Extensibility, L (mm)		Baking strength, S (cm^2)		Elasticity (cm^2)		L/P ratio		Swelling index, G (cc)		Deformation work W (erg)	
	WE	WR	WE	WR	WE	WR	WE	WR	WE	WR	WE	WR	WE	WR
100	43	43	98	98	22.8	22.8	11.0	11.0	2.28	2.28	22.5	22.5	155.6	155.6
97	54	61	78	84	23.4	19.0	14.3	8.5	1.44	1.38	19.8	20.3	155.4	123.2
94	67	69	56	77	23.0	26.1	14.5	14.0	0.84	1.12	16.8	16.9	153.2	159.2
91	77	76	47	53	24.0	26.0	15.0	13.8	0.61	0.83	15.9	17.3	170.2	162.7
88	75	84	46	60	21.2	27.3	11.1	13.7	0.61	0.71	15.2	17.0	140.4	173.2
85	98	99	46	51	28.7	28.8	16.9	15.0	0.47	0.51	15.0	15.6	185.3	181.4
82	109	62	31	43	25.6	17.0	17.5	9.2	0.28	0.69	12.8	15.0	178.6	117.4

WE and WR are as in Table 1.

TABLE 3. EFFECT OF BLENDING ENZYME ACTIVE SOY FLOUR AND ROASTED SOY FLOUR ON BREAD CHARACTERISTICS OF WHEAT FLOUR

Wheat flour in blend (%)	Loaf wt. (g)		Loaf height (cm)		Loaf vol. (cc)		Specific loaf vol. (cc/g)		Protein (%)		Increase in protein (%)	
	WE	WR	WE	WR	WE	WR	WE	WR	WE	WR	WE	WR
100	136.5	136.5	8.3	8.3	400	400	2.98	2.93	8.4	8.4	—	—
97	142.0	137.0	8.2	9.0	390	415	2.75	3.03	9.4	9.2	12.2	9.8
94	147.0	137.8	8.1	8.7	375	405	2.55	2.94	10.8	10.6	29.3	26.9
91	149.3	138.5	8.0	8.6	360	400	2.41	2.89	11.5	11.3	36.9	34.5
88	151.3	141.0	7.5	7.8	340	380	2.25	2.69	12.5	12.1	49.3	44.5
85	151.8	141.5	7.1	7.5	320	360	2.11	2.54	13.4	12.9	59.5	54.6
82	152.0	142.8	6.9	7.2	310	355	2.05	2.49	14.4	14.0	71.9	67.1

WE and WR are as in Table 1.

TABLE 4. EFFECT OF BLENDING ENZYME ACTIVE SOY FLOUR AND ROASTED SOY FLOUR ON ORGANOLEPTIC SCORE OF WHEAT FLOUR BREAD

Wheat flour in blend (%)	General appearance		Crust colour		Grain structure		Texture		Flavour		Overall acceptance		Grade*	
	WE	WR	WE	WR	WE	WR	WE	WR	WE	WR	WE	WR	WE	WR
100	23.5	23.5	9.2	9.2	18.9	18.9	18.7	18.7	23.7	23.7	94.0	94.0	Excellent	Excellent
97	21.2	21.2	8.6	8.7	17.6	17.8	16.9	16.7	20.8	20.7	85.1	85.1	Good	Good
94	19.4	19.6	7.8	8.0	16.0	16.2	15.3	15.8	19.3	19.0	77.8	78.6	Satisfac-tory	Satisfac-tory
91	17.3	17.7	6.9	6.8	15.1	15.6	13.8	14.2	16.5	17.2	69.6	71.5	Satisfac-tory	Satisfac-tory
88	15.6	16.9	5.8	6.7	12.8	14.2	12.2	13.3	16.1	16.6	62.5	67.7	Fair	Satisfac-tory
85	14.1	14.6	5.6	5.8	11.9	12.7	9.9	12.7	13.2	13.5	54.7	59.8	Fair	Fair
82	12.1	12.4	4.6	4.7	10.2	10.7	10.0	10.2	11.7	12.1	48.6	50.1	Poor	Poor

*Grading score—Excellent, 91-100; Good 81-90, Satisfactory, 66-80; Fair, 51-65, and Poor, 50 or less
WE and WR are as in Table 1.

flour. The increase in loaf weight with enzyme active soy flour might be due to greater water absorption as compared to roasted soy flour. The increase in protein content of soy fortified breads was proportionate to the increase in level of soy flour incorporation.

Sensory characteristics of soy fortified bread: The results in Table 4 show the effect of soy flour addition on the sensory characteristics of wheat breads. The sensory qualities such as general appearance, crust colour, grain structure, texture and flavour of soy fortified breads scored lower as compared to control. However, the roasted soy flour fortified bread scored higher than enzyme active soy flour fortified breads. From the sensory evaluation results (Table 4) it can be concluded that a satisfactory bread can be prepared by adding 9 per cent enzyme active soy flour or 12 per cent roasted soy flour.

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Effect of Heat and Germination on Trypsin Inhibitor Activity in Lentil and Pigeon Pea

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Effects of heat and germination on trypsin inhibitor activity have been studied in seven strains of lentil (*Lens culinaris*) viz. 'L-9-12', 'LH-82-3', 'LH-82-4', 'LH-82-6', LH-82-7', 'LH-21' and 'LH-311', and six strains of pigeon pea (*Cajanus cajan*) viz. 'H-77216', 'UPAS-120', 'Prabhat', 'T-21', 'H-77208' and 'H-7244'. Lentil and pigeon pea seeds on an average have similar levels of trypsin inhibitor activity. However, intraspecies variation in activity was wider in pigeon pea. All the heat treatments namely dry heating, autoclaving and heating in boiling water bath, as well as presoaking and germination for six days resulted in a decrease in trypsin inhibitor activity, in lentil and pigeon pea. Heating in boiling water bath was most effective. As compared to pigeon pea, the trypsin inhibitor in lentil was more susceptible to all the treatments except dry heating which was more effective in pigeon pea than in lentil. Varietal differences with regard to various treatments were not of much significance, except for germination of pigeon pea, where maximum loss in trypsin inhibitor activity occurred in 'H-77216' (40%).

Trypsin inhibitor is perhaps the most important protease inhibitor, since trypsin not only activates several of the digestive proteases which are secreted as inactive proenzymes in the digestive tract, but also regulates their secretion[1]. Trypsin inhibitor activity is known to be destroyed by heat; the extent of inactivation de-

pends upon temperature, duration of heating, particle size and moisture conditions[2]. There are several ways in which the material can be subjected to heat treatment viz. normal cooking, quick cooking and dry heating[3], autoclaving[4], atmospheric steaming,[5] microwave heating[6], and dielectric heating[7]. Germination, in general, improves the nutritive value of seeds, although, trypsin inhibitor activity may increase, decrease or remain unaltered[2]. Most of the studies on these aspects of trypsin inhibitor activity have been conducted on soybean[2] and little information is available for other legumes particularly lentil and pigeon pea which are important pulse crops of India. The present communication reports the effects of heat and germination on trypsin inhibitor activity in some of the newly released varieties and promising strains of lentil and pigeon pea.

Materials and Methods

Seeds of seven strains of lentil (*Lens culinaris*) viz. 'L-9-12', 'LH-82-3', 'LH-82-4', 'LH-82-6', 'LH-82-7', 'LH-21' and 'LH-311'; and six strains of pigeon pea (*Cajanus cajan*) viz. 'H-77216', 'UPAS-120', 'Prabhat', 'T-21', 'H-77208' and 'H-7244' were procured from the Department of Plant Breeding, Haryana Agricultural University, Hisar. The variety 'L-9-12' served as a control in lentil. The seeds were freed of foreign material like husk, stones, etc.

Dry heating: The flour samples were heated in a hot air oven at 121 °C for 10 and 20 min (pigeon pea) and one and two hour (lentil). The heated flour was brought to room temperature in a desiccator and stored in a tightly capped container for further use.

Autoclaving: The flour samples were autoclaved for 10 and 20 min at 121 °C (15 psi). The treated flour was dried in a hot air oven at 60 °C and then stored in tightly capped containers until analyzed.

Boiling: One gram seed of each strain was weighed, washed and dropped into 50 ml of boiling distilled water. Boiling was continued for 10 and 20 min. The contents were brought to room temperature, the fluid drained off and the seeds analysed for trypsin inhibitor activity.

Presoaking: One gram seed of each strain was weighed, washed and soaked overnight (24 hr) in 50 ml distilled water. The water was drained off and the seeds used for analysis.

Germination: The seeds of each strain were immersed in 0.2 per cent mercuric chloride for 2 min. The solution was drained off and the seeds were washed 4-5 times with running tap water to remove the residual mercuric chloride. The washed seeds were immersed overnight in excess of distilled water. The soaked seeds were placed on Whatman No. 1 filter paper discs in petriplates and incubated at 28 °C for six days. Chloramphenicol solution (20 ppm) was supplied twice a day in amounts sufficient to keep the seeds turgid, but avoid them being immersed in the solution.

Trypsin inhibitor activity: Trypsin inhibitor activity was determined by assaying trypsin activity in the presence and absence of inhibitor extract, using casein as the substrate for trypsin. The trypsin inhibitor was extracted with phosphate buffer (0.1M, pH 7.6), either by shaking on a mechanical shaker in case of flour, or homogenizing with a pestle in a mortar in case of seeds. A suitable aliquot of extract was used for assaying trypsin inhibitor activity by slight modification of the method described by Roy and Rao[8]. The assay mixture containing 1 ml of phosphate buffer (0.1M, pH 7.6), 0.5 ml trypsin solution (1 mg/ml 0.001 N HCl), 0.5 ml HCl (0.001N), 2 ml casein solution (2 per cent in phosphate buffer, 0.1M, pH 7.6) and 1 ml inhibitor extract was incubated for 20 min in a water bath at 37 °C. The reaction was stopped by addition of 6 ml of 5 per cent trichloroacetic acid. A blank set was prepared by adding trichloroacetic acid before the addition of trypsin solution. A control set was also prepared in which the extract was deleted from the assay mixture and another 2 ml of phosphate buffer (0.1M, pH 7.6) was added in its place. After incubation the contents were filtered through Whatman No. 1 filter paper and the released tyrosine was determined in a suitable aliquot (0.1 ml) of the filtrate by Lowry method[9]. The absorbance was read in Spectronic 20, against the blank set. The trypsin inhibitor activity was determined by substracting the amount of tyrosine released in the experimental set from that in the control set. A unit of trypsin inhibitor activity was arbitrarily defined as the amount of inhibitor causing the release of one μ mole of tyrosine under the conditions of the assay.

The extracts as such did not have any free tyrosine detectable by Lowry method[9], under the conditions of the trypsin inhibitor activity assay.

The samples were not defatted before the assay of trypsin inhibitor activity because they contained very low level of crude fat (approximately 1 per cent).

Results and Discussion

Trypsin inhibitor activity: The trypsin inhibitor activity (TIA) in untreated flour samples varied between 638 and 695 units in lentil, and between 547 and 852 units in pigeon pea. As obvious, the range was quite narrow in lentil, but rather wide in pigeon pea. The variety 'L-9-12' had the maximum TIA whereas 'LH-82-3' had the minimum. In pigeon pea the TIA was maximum in 'Prabhat', and minimum in 'UPAS-120'. Significant genetic variations with regard to TIA have also been recorded in soybean[10]. Both 'L-9-12' of lentil and 'Prabhat' of pigeon pea have been found to be

TABLE 1. EFFECT OF HEAT TREATMENT ON TRYPSIN INHIBITOR ACTIVITY OF LENTIL SEEDS*

Strain	Control	Autoclaving		Dry heating	
		20 min	10 min	2 hr	1 hr
'L-9-12'	695	Nil	75	100	93
'LH-82-3'	638	Nil	75	63	139
'LH-82-4'	642	Nil	128	48	106
'LH-82-6'	695	Nil	88	48	185
'LH-82-7'	692	Nil	46	52	155
'LH-21'	660	Nil	75	63	139
'LH-311'	684	Nil	46	40	117

*μ mol of tyrosine released/g of material. Boiling even for 10 min destroyed trypsin inhibitor activity completely.

Each value in this and subsequent tables, is an average of two replicates.

TABLE 2. EFFECT OF PRESOAKING AND GERMINATION ON TRYPSIN INHIBITOR ACTIVITY OF LENTIL SEEDS*

Strain	Control	Pre-soaking	Germination	
			3 day	6 day
'L-9-12'	695	259	256	100
'LH-82-3'	638	240	216	73
'LH-82-4'	642	221	205	63
LH-82-6'	695	260	250	134
'LH-82-7'	693	268	250	115
'LH-21'	660	252	225	48
'LH-311'	684	289	212	48

*μ mol of tyrosine released/g of material

superior to other strains of respective legumes on the basis of proximate composition[11]. However, Tannous and Ullah[12] could not detect any TIA in lentil seeds. On, the other hand Jaffe[13] reported substantial activity of trypsin inhibitor in lentils, though it was much lower than in other legumes including black kidney bean, red kidney bean, soybean, lima bean and pigeon pea.

Dry heating: Dry heating of lentil flour in air oven at 121 °C for 1 and 2 hr led to progressive decrease in TIA (Table 1). Most of the activity was lost during the first hr of heating (73-87 per cent) and was reduced further to 6-14 per cent during the second hr of heating as compared to the untreated flour. When pigeon pea flour was heated in a similar manner, a substantial decline in TIA took place, even after 20 min of heating (Table 2). Dry heating of winged beans for 30 min at 200°C has been reported to destroy TIA completely[3].

Autoclaving: Autoclaving of lentil flour at 121 °C for 20 min resulted in complete loss of activity in all the strains studied. When the time of autoclaving was reduced to 10 min, TIA ranged from 7 to 20 per cent as compared to untreated flour (Table 1). In pigeon pea, a similar treatment for 10 min resulted in marked loss of activity (Table 2). As much as 88 ('H-77208') to 100 per cent ('UPAS-120') activity was lost when the pigeon pea flour was autoclaved for 20 min. Autoclaving of legume seeds even for 5 min has been found to cause a drastic loss of TIA[3,4]. Although autoclaving of navy beans for more than 5 min led to decrease in TIA, it was also accompanied by a decrease in protein efficiency ratio of the autoclaved flour samples in rats[4].

Boiling: Heating seeds in boiling water even for 10 min destroyed TIA completely in lentil irrespective of the strain (Table 1). Similar treatment of pigeon pea for 20 min decreased the activity to very low values (Table 2). As expected, moist heating enhanced the loss of TIA over dry heating. Moisture appears to mediate some unidentified biochemical reaction accomplishing inactivation of trypsin inhibitors. A decline of 60 per cent in

TABLE 3. EFFECT OF HEAT TREATMENTS ON TRYPSIN INHIBITOR ACTIVITY OF PIGEON PEA SEEDS*

Strain	Control	Autoclaving		Boiling	Dry heating	
		10 min	20 min	20 min	10 min	20 min
'H-77216'	815	76	39	22	142	27
'UPAS-120'	547	80	Nil	107	122	Nil
'Prabhat'	852	54	24	53	192	59
'T-21'	681	77	25	77	126	84
'H-77208'	651	103	81	51	115	116
'H-7244,	700	86	22	48	128	121

*μ mol. of tyrosine released per gram of material.

TABLE 4. EFFECT OF GERMINATION ON TRYPSIN INHIBITOR ACTIVITY OF PIGEON PEA SEED FLOUR*

Strain	Control	Pre-soaking	Germination	
			3 day stage	6 day stage
'H-77216'	815	753	897	488
'UPAS-120'	547	532	721	633
'Prabhat'	852	840	852	799
'T-21'	681	601	739	495
'H-77208'	651	566	655	471
'H-7244'	700	601	470	500

*μ mol tyrosine released/g of material.

TIA has been observed on heating winged bean[3] in boiling water for 20 min.

Presoaking and germination: Presoaking of lentil seeds for 24 hr resulted in 58-66 per cent decrease in TIA, the decrease being most marked in 'LH-82-4' and minimum in 'LH-311' (Table 3). When these presoaked seeds were allowed to germinate, only slight further decrease occurred in TIA at 3 day stage, except in 'LH-311'. Six day old lentil seedlings had substantially lower TIA (21-54 per cent) as compared to 3 day old seedlings (Table 3). However, when pigeon pea seeds were soaked for 24 hr in water, decrease inTIA was not substantial (1-14 per cent) (Table 4). Germination of presoaked seeds caused an increase in TIA at 3 day stage. At six days after germination, there was a larger decrease in TIA, as compared to the increase at 3 day stage, leading to an overall decrease in TIA, as compared to untreated flour samples. The loss in TIA at six day stage was maximum in 'H-77216' (40 per cent) and minimum in 'UPAS-120' in which there was actually an increase in TIA (Table 4). It has been found in mung bean that trypsin inhibitor is rapidly modified by limited proteolysis during the early stages of seedling growth and the modifying activity increases upto 6 days after imbibition[14]. This may explain the marked decrease in TIA observed in lentil. The situation in pigeon pea may, however, be different.

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Utilization of Potato Peels by Fungi for Protein Production

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Out of the ten fungi tested, *Pleurotus ostreatus*, a non-toxic fungus was able to utilize potato peels effectively for maximum protein production by solid state fermentation. The optimum temperature, incubation period, pH, nitrogen source and its level for maximum carbohydrate utilization and protein production by the fungus were 30°C, 17 days, 5.5 and ammonium chloride (0.04% nitrogen w/w) respectively. *P. ostreatus* could increase crude protein of potato from 11.2% to 21.0% and true protein from 7.5% to 11.6%.

Potato (*Solanum* spp.) is the fourth largest world food crop after wheat, maize and rice.[1] It also figures as one of the major vegetable crops of Punjab. Being a seasonal and a perishable crop, gluts are observed during the peak season. However, canneries process the surplus potato crop. While processing potatoes into various products about 20-50 per cent of the raw product is discharged as waste[2] which consists of peels, trimmings. slices, effluents, etc. The discharge of liquid and solid wastes of the processing plant creates pollution problems. According to Shaw[3], at least 5 per cent edible material is lost as waste in potato-processing industries and he suggested its conversion into microbial protein. Dickey *et al.*[4] have suggested utilization of potato waste after fermentation as livestock feed. Due to shortage of protein sources, an attempt has been made in the present study to utilize potato peels for fungal protein production. Several fungi were screened for this purpose.

Materials and Methods

The fungi were maintained on potato dextrose agar (PDA) slants by subculturing fortnightly.

Screening: Twentyfive grams of dried potato peels were mixed with 15 ml distilled water in 500 ml conical flasks and autoclaved at 15 psi for 35 min. The flasks were inoculated with a bit of respective PDA slant and incubated at 25°C for 15 days. The fermented potato peels after incubation were uniformly spread, dried at 70°C for 16-18 hr and ground into fine powder for chemical analysis.

Solid state fermentation of potato peels by P. ostreatus: To 500 ml conical flasks containing 10 g of dried potato peels were added 0, 10, 20, 30 and 40 ml of water and they were autoclaved at 15 psi for 30 min. After cooling, the flasks were inoculated with *Pleurotus ostreatus* inoculum and incubated at 25°C for 15 days. After incubation the samples were dried in the oven at 70°C to constant weight and ground for chemical analysis. Thirty ml water/10 g substrate was found optimum for degradation of carbohydrate and maximum increase in protein. The effect of various cultural conditions, viz., temperature (0-40°C), incubation period (0-21 days), pH of 3.0 to 7.0 and different nitrogen sources like, ammonium chloride, urea, ammonium sulphate, calcium nitrate and potassium nitrate at 0-0.1 per cent nitrogen (w/w) level, were studied to find optimum conditions for maximum biomass production.

Estimation and chemical analysis: The utilized carbohydrates of the fermented potato waste and total carbohydrates of the unfermented substrate were estimated by the phenol sulphuric acid method of Norris and Ribbon[5]. Total nitrogen was determined by micro Kjeldahl[6] method. After precipitation with 10 per cent trichloroacetic acid and redissolving in 0.66 N sodium hydroxide true protein was estimated according to Lowry *et al*[7].

All determinations were done in triplicate and averages are given.

Results and Discussion

Out of ten fungi screened, six could grow on potato peels (Table 1). *P. ostreatus* showed maximum utilization of carbohydrates (38.2 per cent, residual) and produced highest crude and true protein (14.9 and 12.0 per cent respectively) closely followed by *Aspergillus niger*. *Polyporous versicolor* produced lowest quantity of crude and true protein (11.6 and 8.1 per cent respectively). Due to the non-toxic nature, *P. ostreatus*, was selected for further studies. Variation in the performance of other fungi used for screening may be due to different nutritional requirements, sub-optimal moisture content and inability to utilize the starch of potato peels.

TABLE 1. PROTEIN ENRICHMENT BY DIFFERENT FUNGI USING POTATO PEELS

Organism	Carbohydrates (residual)	Protein	
		Crude (%)	True (%)
Agaricus bisporus	—	—	—
Aspergillus niger	39.1	14.6	11.5
Chaetomium cellulolyticum	—	—	—
Coprinus sp.	—	—	—
Fusarium moniliforme	40.0	14.3	10.0
Myrothecium verrucaria	51.0	12.0	8.7
Pleurotus ostreatus	38.2	14.9	12.0
Polyporous versicolor	45.0	11.6	8.1
Sporotrichum pulverulentum	—	—	—
Trichoderma koningii	50.0	12.0	9.0

(—)=No growth
Water content=60 ml/100 g of potato peels.
Incubation temperature=25°C.
Raw potato peel contained 53.0% carbohydrate, 11.2% crude protein and 7.5% true protein.
Values are on dry matter basis.

Moreton[8] showed that growing of *Candida utilis* on potato waste was only partially successful due to poor amylase production by *Endomycopsis* spp. Lines[9] introduced *Endomycopsis fibuliger* into the growth medium consisting of potato processing waste to produce amylase for breaking down starch to glucose and *Candida utilis* used the glucose for growth. Lemmel[10] propagated *C. utilis* and *Saccharomyces fibuliger* on potato processing waste water for single cell protein production.

There was negligible growth of *P. ostreatus* at 0°C. (Table 2). The optimum temperature for growth of the fungus was 30°C; the residual carbohydrates at this temperature were least (21 per cent) and crude and true protein produced were highest (20.0 and 13.9 per cent respectively). At 40°C the growth of the fungus was profuse and powdery white. These observations agree with those of Kahlon and Nikhat Parveen[11] who found 30°C as the optimum temperature for growth of *P. ostreatus* on wheat straw. Rangad and Jandaik[12] also found 25-30°C as the optimum temperature for *P. ostreatus*, *P. eryngii* and *P. cornucopiae* grown on Dimmick solution. Jauhri *et al.*[13] found 27°C as the optimum temperature for *P. ostreatus* grown on sugarcane bagasse.

TABLE 2. EFFECT OF INCUBATION TEMPERATURE ON THE UTILIZATION OF CARBOHYDRATES AND PRODUCTION OF PROTEIN FROM POTATO PEELS BY *P. OSTREATUS*

Incubation temp (°C)	Carbohydrates (residual)	Protein	
		Crude (%)	True (%)
0	49.0	11.0	8.0
20	28.0	17.0	12.0
25	26.0	17.7	13.6
30	21.0	20.0	13.9
40	41.6	13.0	10.3

Water content: 30 ml/10 g of potato peels
Incubation period is 15 days.
Values are on dry matter basis.

TABLE 3. EFFECT OF INCUBATION PERIOD ON CARBOHYDRATES AND PROTEIN CONTENT OF FERMENTED POTATO PEELS BY *P. OSTREATUS*

Incubation period (days)	Carbohydrates (residual)	Protein	
		Crude (%)	True (%)
0	51.0	11.8	7.5
3	35.0	12.5	8.1
7	30.0	18.0	12.7
13	24.0	18.5	13.5
17	21.0	19.0	14.0
21	20.0	16.8	11.2

Water content: 30 ml/10 g of potato peels
Incubation temperature: 30°C.
Values are on dry matter basis.

As the incubation period was prolonged (Table 3), carbohydrate utilization also increased. Carbohydrates were reduced from 53 to 20 per cent in 21 days. But the production of crude and true protein was not maximum at 21 days of incubation. The decrease in protein content might be due to degradation of some of the organic nitrogen by the fungus. Both crude and true protein increased upto 17 days of incubation at which time they were 19 and 14 per cent respectively. Similarly, 14 days were required by *P. ostreatus* to produce maximum biomass when grown on bench wood as reported by Tsao[14] and on sugarcane bagasse as observed by Jauhri *et al.*[13] Khanna[15] reported 12 days incubation for maximum yield of *P. ostreatus* grown on mannose in the medium, though he obtained highest protein content on the 8th day of incubation. Hence the incubation period for efficient growth of fungus depends on the type of the media and particularly the carbon source used.

Kahlon and Nikhat[11] reported that ammonium chloride was the best for *P. ostreatus* for maximum degradation of lignin and cellulose of wheat straw. However, Jauhri[16] found urea to be the best nitrogen source for *P. ostreatus* grown on bagasse. In the present

TABLE 4. EFFECT OF VARIOUS NITROGEN SOURCES ON THE CARBOHYDRATE AND PROTEIN CONTENT OF FERMENTED POTATO PEELS BY *P. OSTREATUS*

Nitrogen source	Carbohydrates (residual)	Protein	
		Crude (%)	True (%)
Control	28.8	16.3	11.7
Ammonium chloride	21.8	18.8	13.7
Urea	24.5	18.5	13.5
Ammonium sulphate	25.1	18.2	12.5
Calcium nitrate	25.7	17.8	11.3
Potassium nitrate	26.5	17.8	11.0

Nitrogen sources added to provide 0.04% N (w/w).
Values are on dry matter basis.

investigation, ammonium chloride was found to be the best nitrogen source for *P. ostreatus* in utilizing maximum carbohydrates, where it was reduced to 21.8 per cent as compared to 28.8 per cent reduction observed in fermented potato waste with no nitrogen supplementation. Ammonium chloride also increased crude and true protein to 18.8 and 13.7 per cent respectively, closely followed by urea (Table 4). Table 5 shows that with increase in the amount of ammonium chloride beyond 0.04 per cent nitrogen carbohydrate utilization was not further enhanced. However, there was a significant increase in crude and true protein with increase in concentration of ammonium chloride. These findings are contrary to Zadrazil and Brunnert[17], who reported no correlation between lignin degradation and nitrogen supplementation in case of *P. ostreatus* var. Florida. Kahlon and Nikhat[11] observed increase in growth of *P. ostreatus* upto 0.8 g nitrogen/l in the form of calcium nitrate and Kahlon and Das[18] found 0.08 per cent nitrogen in the form of ammonium chloride best for *P. ostreatus* on rice straw. Thus, there is a wide variation in potentialities of various fungi to utilize crop residues with supplementation of different nitrogen sources. The nitrogen sources viz. urea and ammonium chloride were equally suitable for hastening carbohydrate utilization during 17 days fermentation.

TABLE 5. EFFECT OF DIFFERENT CONCENTRATIONS OF AMMONIUM CHLORIDE ON THE CARBOHYDRATE AND PROTEIN CONTENT OF POTATO PEELS FERMENTED BY *P. OSTREATUS*

NH_4Cl concn (% N w/w)	Carbohydrates (residual)	Protein	
		Crude (%)	True (%)
0	28.8	17.0	11.0
0.02	25.0	18.0	12.0
0.04	21.9	19.7	14.2
0.06	28.2	17.5	11.8
0.08	25.0	18.2	12.2
0.10	28.0	21.0	14.0

TABLE 6. EFFECT OF pH ON THE CARBOHYDRATE AND PROTEIN CONTENT OF POTATO PEELS FERMENTED BY *P. OSTREATUS*

pH	Carbohydrates (residual)	Protein	
		Crude (%)	True (%)
Control	53.0	11.2	7.5
3.0	49.0	11.9	8.9
4.0	28.0	17.0	12.5
5.0	26.0	17.6	13.2
5.5	21.0	19.6	14.3
6.0	20.0	17.8	13.1
6.5	24.0	16.9	12.8
7.0	35.0	12.6	8.8

The effect of pH on utilization of the substrate by *P. ostreatus* (Table 6) indicates that best results were obtained at pH 5.5 which agrees with the results reported by Wasilewska and Trozanowski[19]. Crude and true protein produced was 19.6 and 14.3 per cent respectively, at pH 5.5. Beyond pH 5.5, both crude and true protein showed insignificant increases. Table 7 shows comparison between control and potato waste enriched under optimum cultural conditions found suitable for solid substrate fermentation. *P. ostreatus* could increase crude

TABLE 7. CHEMICAL COMPOSITION OF UNFERMENTED POTATO PEELS AND FINAL FERMENTED PRODUCT PREPARED UNDER OPTIMUM CONDITIONS

Constituents	Potato peels	
	Unfermented control	Fermented by *P. ostreatus*
Total carbohydrates	53.0	20.1
Crude protein	11.2	21.0
True protein	7.5	14.6
Total ash	10.1	15.8
Crude fibre	—	8.5
Cellulose	—	8.0

Optimum conditions: Water content 30 ml/10g; substrate, NH_4Cl (0.04% N w/w); temp: 30°C; pH: 5.5 and incubation for 17 days.
Per cent carbohydrate utilized was 62%.
Values are on dry matter basis.

protein from 11.2 to 21.0 per cent and true protein from 7.5 to 14.6 per cent.

These experiments provide useful information with regard to culture conditions for maximum utilization of carbohydrates by *P. ostreatus* from potato waste. However, more detailed studies are required for application on a large scale.

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'Zahdi' Dates as a Source of Sugar

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A method was developed in our laboratory to extract sucrose from 'Zahdi' dates using aqueous ethanol. A semi-pilot plant was designed to use this method. It consisted of four major steps: aqueous ethanol extraction, filtration, evaporation and crystallization. Except ethanol no other chemicals or resins were used. Recovery of sucrose was 24.4 and 10.3% of the total wet weight of dates at half and full maturity, respectively. In order to test the efficiency of the method, recovery experiments were carried out in the presence of varying quantities of reducing sugars.

Iraq is one of the major date producing countries in the world. According to the Department of Agricultural Statistics[1], the total production of dates for 1977 season was 578,000 tons, of which 371,000 tons was 'Zahdi' dates.

'Zahdi' dates (*Phoenix dactylifera* L.) contain on dry weight basis approximately 78 per cent sugar comprising glucose, fructose and sucrose[2,3]. At present, dates are used as a source of liquid sugar in Iraq. Because of the

increasing demand for crystalline sugar, dates might be considered as a sugar crop complementary to sugar beet and sugar cane used at present.

Industrial methods used for the production of sugar from sugar cane or beet require high purity juice[4]. Ash content and colouring material should also be minimal[5]. The presence of glucose, fructose, ash and colouring matter in large quantities in dates would decrease the yield of the recovered sucrose; extraction of sucrose would therefore not be possible[6]. In order to overcome such difficulties an adsorbent consisting of a carbonaceous pyropolymer was used by Landis[7] for selective adsorbtion of sucrose from molasses. Large scale production of sugar using this method would be very expensive. Many solvents have also been used for crystallization of pure sucrose and other sugars on a laboratory scale[8]. Crystallization of sucrose from biological samples would be complicated by the presence of other sugars.

This paper describes a method developed in our laboratory for selective crystallization of sucrose from 'Zahdi' dates[9] and its application on a semi pilot scale.

Materials and Methods

Samples: The 'Zahdi' dates were of the 1982 season harvested from Za 'afarania Experimental Station at half and fully-matured stages corresponding to 18 and 15 per cent moisture content respectively.

Sugar analysis: Gas-liquid chromatographic analysis of sugars was performed after trimethylsilyl (TMS) derivatization as reported earlier[3]. Water and aqueous ethanol (70,80,90 and 95 per cent) were used for the extraction of sugars from dates prior to chromatographic analysis. Determinations were conducted in triplicates and variation between samples was minimal.

Laboratory procedure for extraction and crystallization of sucrose from dates: After destoning, each fruit was longitudinaly divided into four parts. Such samples were homogenised with boiling 95 per cent aqueous ethanol (1:5, w/v) using a high speed homogenizer (18,000 rpm) for 10 min followed by filtration using Buchner filters (15-40 μ pore size). The pH of the aqueous extract was 6.1. The filtrate was concentrated into a syrup using a rotary evaporator at 50°C and reduced pressure. Similar extractions were done with water or 90,80, and 70 per cent aqueous ethanol.

Freshly boiled aqueous ethanol (97 per cent) was added (1:12, w/v based on the original sample weight) to the syrup with continuous stirring followed by filtration (Buchner filters 5-15 μ pore size) to remove the fine residues carried over from the first extraction step. The mixture was then heated to just boiling, followed by gradual cooling to room temperature. Scratching the inside wall of the glass flask caused the sucrose, but not other sugars, to precipitate as a fine white powder. It was then recovered by filtration. Evaporation of the filtrate gave glucose-fructose syrup. All the analyses were run in ten replicates.

Semi pilot-scale production of sucrose from dates: The semi-pilot-scale process consisted of four major steps: (*i*) extraction, (*ii*) filtration, (*iii*) evaporation and (*iv*) crystallization. Destoned 'Zahdi' dates (1 kg) were extracted with 95 per cent aqueous ethanol 1:5, (w/v). The extraction vessel was made of stainless steel connected to a reflux condenser with 12m heat transfer area. Variable speed agitator (400 rpm) and stainless steel stirrer with sharp cutting edges were used to liquefy the fruits.

Two stage filtration system was used to separate the juice from the pulp. The filtered juice was pumped to the evaporation vessel. Evaporation was carried out under reduced pressure (600 mm Hg) in a steam jacketed vessel with a reflux condenser. Variable low speed agitator was used for mixing the juice during evaporation. Aqueous ethanol 97 per cent was added 1:12 (w/v) with continuous heating and agitation followed by filtration. The juice was then pumped to the crystallization vessel.

The crystallization vessel was double jacketed with a reflux condenser and a variable low—speed scraped—surface agitator. The mixture was heated to just boiling with continuous agitation, followed by gradual cooling to room temperature. Scratching the inside wall of the vessel with the scrapped-surface agitator caused the sucrose to precipitate as a fine white powder, which was then recovered by filtration. Evaporation of the filtrate gave glucose-fructose syrup.

Results and Discussion

Table 1 shows the quantities of sucrose and other sugars extracted from 'Zahdi' dates with each of four solvent systems Extraction with 95 per cent ethanol yielded white coloured high purity sucrose.

TABLE 1. EXTRACTION (G/100 G WET BASIS) OF SUGARS AND RECOVERY OF SUCROSE FROM 'ZAHDI' DATES

Concn of ethanol (%)	Fructose	α-Glucose	β-Glucose	Sucrose	Sucrose recovered[b]
95	10.74	13.20	8.46	10.70	10.30
90	20.40	11.40	9.24	13.62	10.56
80	14.10	10.80	6.60	12.60	10.45
70	16.20	15.40	8.28	12.60	5.28

[b] Recovered sucrose as white fine powder.

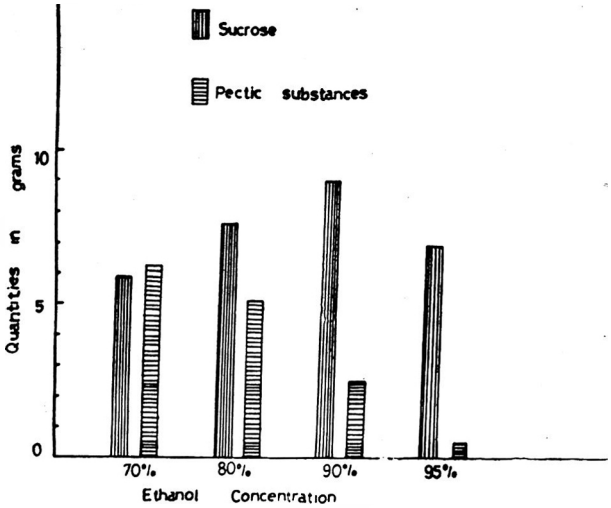

Fig 1. Quantities of sucrose and pectic substances recovered from 25 g of dates after extraction with 70, 80, 90, and 95% ethanol.

Fig 1 shows quantities of sucrose and pectic substances recovered from 25 g dates with 70,80,90 and 95 per cent ethanol. Although the recovery of sucrose was not maximized using 95 per cent ethanol, quantity of pectic substances extracted was minimal.

Fig 2 shows chromatograms of sugars extracted with water at different temperatures compared with that extracted with boiling 95 per cent ethanol. The maximum peak area for sucrose was obtained when extracted with 95 per cent ethanol. Boiling water seemed to provide considerable inhibition to the action of the invertase enzymes but not complete inhibition. The sucrose peak disappeared completely when water at 50°C was used for extraction. This was in agreement with the data reported by Marouf and Zeki[10] who have found the optimum temperature for the activity of soluble and insoluble invertases to be 45°C, insoluble invertase remained active even at 60°C. Li and Schuhmann[11] had earlier reported that extraction with water would lead to extensive hydrolysis of sucrose.

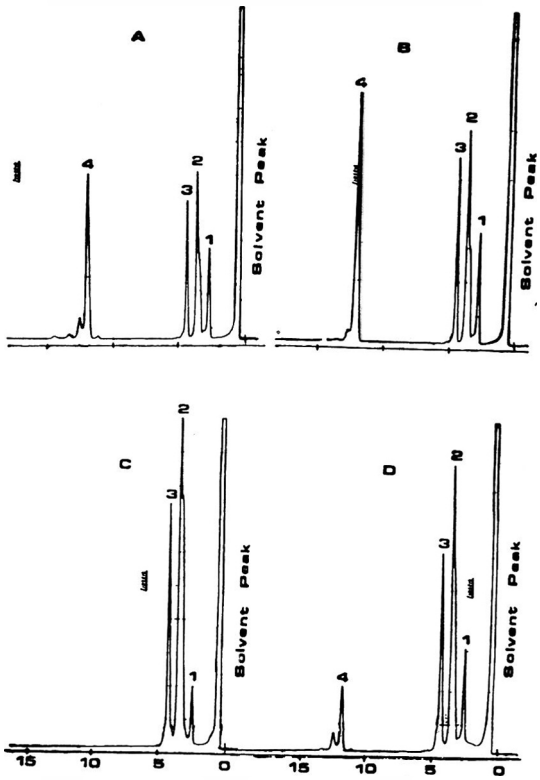

Fig 2. Chromatograms of date sugars extracted with A. boiling water, B. 95% ethanol, C. water at 50°C, D. water at 25°C. Peak identities 1. Fructose, 2. ∝-Glucose, 3. β-Glucose, and 4. Sucrose.

Recovery experiment was carried out for pure sucrose in the presence of varying quantities of reducing sugar (1:1 mixture of glucose and fructose), following the same method used for extraction of sucrose from dates. The recovery of pure sucrose was 92 per cent but became 82.4 per cent when 16.7 per cent reducing sugar was added. When 66.7 per cent of reducing sugars was added, only 31.5 per cent of the sucrose was recoverable.

The major constituents of dates vary according to the stage of ripening. Table 2 shows the per cent of recovered sucrose from date harvested at five different periods from the same location; maximum recovery of 24.4 per cent of sucrose was observed when most of the date samples were at the half mature stage. At the fully matured stage, a drop in sucrose content was noticed and only 10.3 per cent was recoverable. This was mainly due to the high activity of the invertases at the end of maturation stage and partly due to changes in other date constituents.

A semi-pilot-scale plant was designed according to the method developed in our laboratory using ethanolic extraction. Fig 3 represents an outline of the process showing the four major steps: extraction, filtration, evaporation and crystallization.

TABLE 2. EFFECT OF MATURITY ON SUCROSE RECOVERY

Harvestng date	Maturity stage	Seeds (%)	Edible[a] portion (%)	Fibre (%)	Sucrose recovery (% of whole fruit)
11- 9-82	half	14.0	28.0	32.4	24.4
18- 9-82	half	14.2	30.4	36.0	14.1
25? 9-82	half	13.9	35.2	37.0	13.3
2-10-82	full	12.8	35.2	48.0	7.0
9-10-82	full	12.0	32.0	44.0	10.3

a Glucose-fructo e syrup recoveied after the extraction of sucrose.

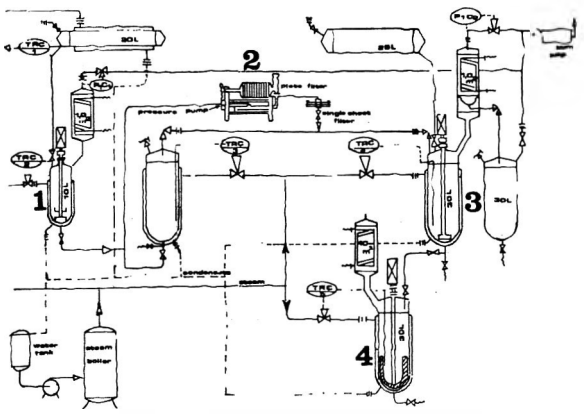

Fig. 3 Outline of a semi-pilot-scale plant for the production of sucrose from 'Zahdi' dates using solvent extraction technique, and consisting of four major steps; 1. Extraction. 2. Filtration. 3. Evaporation, and 4. Crystallization.

In conclusion, 'Zahdi' dates were found to be a good source of crystalline sugar. Other date varieties such as 'Daglat Nuur', grown in North Africa and the United States and containing 38 per cent sucrose[2] could also be used for this purpose.

The method used for extraction of sucrose from dates on a laboratory and semi-pilot-scale using 95 per cent ethanol for extraction 97 per cent ethanol for crystallization was successful. In spite of the presence of large quantities of glucose, fructose, colouring material, and ash in dates the sucrose recovery per cent of whole fruit was 24.4 at half matured stage. The solvent used is nontoxic, recoverable, and could be obtained easily. The recovered sucrose was a white fine powder.

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Oryzanol Content of Indian Rice Bran Oil and Its Extraction from Soap Stock

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The oryzanol content of rice bran oil samples ranged from 1.1-2.6%. The soap stock obtained by alkali refining of the oil contained 1.3-3.1% oryzanol. The optimum pH for extraction of oryzanol from soap stock was around 9.5 and diethyl ether was the best solvent. The oryzanol concentrate could be further upgraded by alumina column chromatography and recrystallized using methanol and methanol-acetone (2:1). The recrystallized material (98% pure) had a melting point of 133-139°C and absorption maxima at 314, 290 and 230 nm in petroleum ether.

A substance having a melting point of 137.5-138.5°C and absorption maxima at 315, 291 and 231 nm was first separated from rice bran oil by Kaneko and Tsuchiya[1]. Assuming the substance to be a single compound they named it as 'oryzanol'. Subsequently oryzanol was shown to be a mixture of several compounds consisting

mainly of ferulic acid esters of triterpenoid alcohols[2]. In recent years there has been considerable interest in oryzanol due to its many possible pharmacological uses like growth accelerating action in animals[3], regulation of estrous cycle as demonstrated in rats[4], and ability to promote skin capillary circulation[5]. Oryzanol is also reported to have anti-itching and anti-dandruff action[6] and has been used in cosmetics[7] and dentifrice[8] preparations. It has been shown to be a good antioxidant for oils and fats[9].

Oryzanol has been reported to be present to the extent of 1.5 to 2.9 per cent in rice bran oil[10]. The processes for extraction of oryzanol from rice bran oil have been covered by patents[11-15]. In this paper the oryzanol content of commercial solvent extracted rice bran oil and the method for extraction of oryzanol from soap stock are reported.

Materials and Methods

Solvent extracted rice bran oil samples were procured from local solvent extraction units. Oil was also obtained by extracting rice bran with hexane and a mixture of chloroform-methanol[16]. All the reagents used were of analytical grade.

Preparation of soap stock: Soap stock from rice bran oil was prepared as follows: The free fatty acids (FFA) content of rice bran oil was determined according to the AOCS procedure[17]. The oil (1 kg) was taken in a 2 l beaker and heated to 65°C. The calculated amount of sodium hydroxide solution (20 per cent) was added to the hot oil with stirring for 20 min. The mixture was allowed to cool and left overnight to settle. The supernatent was decanted and the sediment was centrifuged to obtain solid soap stock.

Estimation of oryzanol: oryzanol content of the oil was estimated by determining optical density of the oil in petroleum ether (60-80°C) at 315 nm and using specific extinction coefficient $E^{1\%}_{1cm}$=358.9.

For measuring the oryzanol content of soap stock, 5 g sample was dispersed in 100 ml water and pH adjusted to 5.5 using 1N HCl. The solution was extracted with diethyl ether till the ether extract became colourless (75 ml×3). The combined ether extract was washed with water till free of HCl, dried over anhydrous sodium sulphate and evaporated on a water bath. The residue was taken in petroleum ether (60-80°C) and the optical density was read at 315 nm.

Preparation of oryzanol concentrate: In the preliminary studies oryzanol was extracted according to the procedure of Tsuchiya *et al.*[19] To 10 g of soap stock 100 ml of water was added and warmed on a water bath for uniform dispersion. This aqueous suspension was cooled and taken in a 500 ml separatory funnel; 25 ml of methanol and 150 ml of ether were added and contents shaken. The ether phase was transferred to another separatory funnel and shaken with 10 ml portions of 5 per cent KOH until the KOH layer became colourless. The KOH extracts were pooled, neutralized with 1N acetic acid and extracted with diethyl ether (50 ml×3). The combined ether extract was washed with water, dried over anhydrous sodium sulphate and solvent evaporated to get oryzanol concentrate.

Effect of pH of soap stock on oryzanol recovery: To assess the effect of pH on oryzanol recovery, the pH of soap stock was adjusted prior to extraction of oryzanol. Soap stock (10 g) was dispersed in 100 ml of water and the pH adjusted to 8.0, 8.5, 9.0, 9.5, 10.0 and 10.5 using 1N HCl or saturated sodium carbonate solution as required. From each of these oryzanol was extracted with diethyl ether as described above.

Isolation and purification of oryzanol: A concentrate containing 16.3 per cent oryzanol was used. The concentrate (1 g) was dissolved in 15 ml of hexane and applied onto a column loaded with neutral alumina (activated at 140°C for 3 hr). The column was eluted in the following order; 50 ml of hexane, 30 ml of petroleum ether-methanol (9:1) and finally with 50 ml of diethyl ether-glacial acetic acid (20:1).

Thin layer chromatography (TLC): TLC was carried out on 250μ thick silica gel G plates activated at 110°C for 1 hr. The sample in chloroform was spotted and the plate was developed first in hexane-diethyl ether (9:1) and then in benzene-diethyl ether (4:1) in the same direction[18]. Oryzanol was located under UV light as blue fluorescent spots.

Results and Discussion

Oryzanol content of commercial rice bran oils (Table 1) ranged from 1.3 to 1.9 per cent The oil extracted from

TABLE 1. ORYZANOL CONTENT OF RICE BRAN OIL AND SOAP STOCK

Rice bran oil		Soap stocks	
Sample	Oryzanol (%)	Sample No.	Oryzanol (%)
Commercial	1.52	1	3.14
	1.34	2	2.23
	1.95	3	2.32
	1.57	4	2.20
	1.93	5	1.30
Hexane extracted	1.33		
	1.12		
	1.38		
Chloroform: methanol (2:1) extracted	2.61		
	2.55		

TABLE 2. EXTRACTABILITY OF ORYZANOL FROM SOAP STOCK USING DIETHYL ETHER AND ACETONE AT DIFFERENT pH

pH	Diethyl ether			Acetone		
	Wt. of conct.[a] (g)	Oryzanol content of conct.[b] (%)	Recovery* (%)	Wt. of conct.[a] (g)	Oryzanol content of conct.[b] (%)	Recovery* (%)
8.0	2.79	3.7	79.4	2.11	4.9	79.5
8.5	1.35	7.5	77.9	1.77	5.7	77.6
9.0	0.61	16.3	76.5	1.42	7.2	78.6
9.5	0.37	24.0	68.3	0.84	10.5	67.8
10.0	0.27	31.6	65.6	0.34	17.5	45.7
10.5	0.19	36.6	53.5	0.20	26.3	40.5

a from 10 g soap stock containing 1.3% oryzanol

* Recovery $= \frac{a \times b}{0.13}$

rice bran in the laboratory using hexane had oryzanol content ranging between 1.1 and 1.4 per cent. These values are lower than what is reported (1.5-2.9 per cent) for Japanese rice bran oils[10]. Chloroform-methanol (2:1) extracted rice bran oil had higher oryzanol content (2.55-2.61 per cent).

Effect of pH of soap stock on the extraction of oryzanol: Data in Table 2 show that as the pH of the soap stock increased from 8.0 to 10.5 the oryzanol content of the concentrate increased. However, at higher pH, specially above 9.5 there was problem of emulsion formation. Also the recovery of oryzanol decreased as the pH of the soap stock increased. A pH of around 9.5 was therefore considered optimum for extraction of oryzanol.

Suitability of various solvents for oryzanol extraction: Oryzanol is soluble, in addition to ether, in solvents like acetone and alcohol. It is soluble with difficulty in non-polar solvents like petroleum ether and hexane[19]. Hence the suitability of these solvents for extraction of oryzanol from soap stock was investigated. Hexane and alcohol were not suitable due to emulsion formation and complete dissolution of the soap stock respectively.

Acetone was as good as diethyl ether for extraction of oryzanol from soap stocks of upto pH 9.5 (Table 2). However, the oryzanol content of the acetone extract was considerably lower than that of diethyl ether extracts.

Isolation and purification of oryzanol: Column chromatography on alumina was helpful in enriching the concentrate to the extent of 51 per cent (Table 3). Oryzanol from this concentrate was crystallized using methanol and recrystallized using methanol-acetone (2:1). It had a melting point ranging from 133 to 139°C.

TABLE 3. ISOLATION OF ORYZANOL BY COLUMN CHROMATOGRAPHY USING NEUTRAL ALUMINA

Fractions eluted with	Wt. of fraction[a] (g)	Oryzanol content of fraction[b] (%)	Recovery* (%)
Hexane (50 ml)	0.10	0.2	0.1
Petroleum ether-methanol (9:1) (30 ml)	0.61	5.8	21.7
Diethyl ether-acetic acid (20:1) (50 ml)	0.24	51.4	75.7

a from 1g concentrate containing 16.3% oryzanol.

* Recovery $= \frac{a \times b}{0.163}$

This recrystallized material showed absorption maxima at 314, 290 and 230nm in petroleum ether as compared with 315, 291 and 231 nm in heptane reported in literature[19].

The TLC of the recrystallised oryzanol on exposure to iodine vapours showed 2 spots. (Rf 0.55 and 0.65) which exhibited blue fluorescence under UV light. These spots also answered the confirmatory tests for ferulates[18] showing pink spots on yellow background and appearing blue under UV light when sprayed with aqueous solution of 0.04 per cent fluorescein sodium and showed purple spots when sprayed with a saturated solution of antimony trichloride in chloroform and heating at 110°C.

Acknowledgement

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Oxidative Rancidity in Groundnut Oil—Evaluation by Sensory and Chemical Indices and Their Correlation

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Stored and commercial groundnut oil samples were tested for sensory parameters of odour and flavour along with chemical values to explore any functional relationship between them. Kreis test was found to be a better predictor of early stages of rancidity than peroxide value because of higher slope of regression though both were promising. Further, peroxide value of 10 as recommended by Codex Alimentarius was not valid for evaluating quality in groundnut oil.

Quality evaluation in some foods is conducted meaningfully when the flavour impact components are chemically analysed[1]. Rancid flavour in oil is known to occur due to oxidation and no single compound is responsible for it, but sensorily perceived rancidity is clear and gives a direct indication of quality.[2] These sensory techniques have been recognized[3] and continued to be found essential for odour evaluation in oil[4]. Correlations were, therefore, attempted between common chemical methods and sensory analysis in the early fifties[5-7]. Later with the

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advent of gas charmatography selected derivatives[8] or whole GC profile have been used for correlation with sensory rancidity or pleasantness[9-11]. Correlations were worked out with highly significant coefficients for predictive purpose with linear relationship[8-11]. However, the sensory test methods are still found to lack standard methodology with each worker using different temperatures of testing, sample size[5,7,12] sensory methodology[3] and scale[4-7,11].

Among the common chemical tests used for groundnut oil peroxide value (PV) is commonly fixed at a limit of 10 as indicative of quality by the Codex Alimentarius. Other chemical tests like anisidine value (AV) and Kreis value (KV) are also used to evaluate quality in commercial samples of edible oils. The suitability of these methods to monitor rancidity has been studied earlier[14].

Groundnut oil is the major edible oil in India, constituting about 45 per cent of the total edible oils. The present study was aimed at quantification of sensory response to rancidity development in raw groundnut oil through appropriate methodology of sensory testing and exploring the relationship between chemical and sensory responses under Indian climatic and storage conditions.

Materials and Methods

Raw groundnut oil was stored at ambient (20-27°C), 30 and 37°C in closed tins, maintaining constant surface to volume ratio and head space. Sensory and chemical analyses were done at 4th week, 8th week and every second week thereafter till 18th week for samples stored at ambient and 30°C temperatures. Samples stored at 37°C were tested at every 2nd week from the beginning till the 18th week.

Twelve commercial samples collected from both small scale and large scale retailers were used in the study.

Chemical methods: Peroxide value was estimated by AOCS method[14], Kreis test by modified photocolorimetry[15] and anisidine value by the modified Jirousova method[16].

Sample preparation: For odour test 10 ml of test samples were left overnight in small wide mouthed glass stoppered bottles (35 ml capacity) allowing for about 1/3 head space volume. For flavour tests, samples were prepared as emulsion (10 per cent) in water using a mechanical emulsifier and 10 ml was presented. The emulsion was stable upto three hr. Odour and flavour evaluations were done with samples at room temperature since normal commercial testing is done at this temperature.

Development of score card: The test was designed to obtain information on freshness of oil, identification of rancidity and also of possible incipient rancidity. Hence, four stage quality parameter, 1-fresh and typical, 2-bland indicative of incipient rancidity, 3-slightly rancid and 4-clearly rancid, were used in evaluation of both odour and flavour. Comments on any other defects were also asked. Table 1 gives the score card used. Puffed rice or bread was used in between samples in addition to warm water (40-50°C) for neutralisation of palate. A maximum of four samples were tested per session.

TABLE 1. SCORE CARD FOR SENSORY EVALUATION OF GROUNDNUT OIL

Name:..... Date:.....

I. Odour evaluation

- Smell the sample and put a (√)at the appropriate place for its odour quality.
- Wait for a few minutes before proceeding to the next sample.

Code No.	Typical fresh	Bland (no rancidity)	Slightly rancid	Clearly rancid

II. Flavour evaluation

- Taste spoonful of sample and put a (√) at the appropriate place for its flavour quality.
- Rinse your mouth with warm water before proceeding to the next sample.

Code no.	Typical fresh	Bland (no rancidity)	Slightly rancid	Clearly rancid

Panel selection and training: Since the aim of the test was to identify rancidity perception at use level and relate it to chemical tests, panelists were drawn from among the scientific and technical staff of the laboratory. They were, however, familiar with sensory evaluation methods of testing. To reduce ambiguity in understanding different stages of rancidity, preliminary training sessions were held. Samples with different degrees of rancidity were prepared in the laboratory and used in these training sessions. Attention was drawn to minor differences.

Test conditions: Sensory tests were conducted as specified under Indian Standards[17] and in the forenoon.

TABLE 2. MEAN VALUE OF SENSORY SCORES FOR QUALITY OF GROUNDNUT OIL

Weeks	Odour score*			Flavour score*		
	Ambient	30°C	37°C	Ambient	30°C	37°C
0	1	—	—	1	—	—
2	1	—	1.2	—	—	1.1
4	1.1	1.0	1.3	1.3	1.3	1.3
6	—	—	1.3	—	—	1.3
8	1.5	1.3	1.4	1.4	1.3	1.2
10	1.8	1.3	1.5	1.5	1.5	1.3
12	2.0	1.6	1.6	2.2	1.5	1.8
14	2.0	1.8	1.9	2.2	1.8	2.3
16	2.0	2.0	1.9	2.0	2.0	3.0
18	2.2	2.1	2.2	2.1	2.1	3.0

*Scale 1=Typical and fresh groundnut oil
2=Blank-indicates incipient rancidity
3=Rancidity just noticeable
4=Rancidity clearly noticeable.

TABLE 3. CHEMICAL AND SENSORY QUALITY ANALYSIS OF GROUNDNUT OIL STORED AT DIFFERENT TEMPERATURES

Weeks	Peroxide value	Kreis value	Anisidine value	Sensory Score* odour	flavour
			Initial		
0	1.5	0.25	22	1	1
			Ambient		
4	4.3	0.30	20	1.1	1.3
8	4.7	0.34	23	1.5	1.4
10	6.9	0.38	29	1.8	1.5
12	11.0	0.40	49	2.0	2.0
14	11.0	0.73	49	2.0	2.3
16	14.0	0.70	72	2.0	2.0
18	16.8	0.69	101	2.2	2.3
			30°C		
4	5.0	0.30	25	1.0	1.3
8	8.2	0.41	44	1.3	1.3
10	15.2	0.76	67	1.3	1.5
12	22.0	0.60	106	1.6	1.3
14	22.0	1.00	106	1.9	2.6
16	29.7	1.30	232	2.0	2.0
18	39.4	1.57	259	2.2	2.3
			37°C		
2	1.7	0.26	21	1.2	1.1
4	5.9	0.34	40	1.3	1.3
6	6.8	0.39	61	1.3	1.3
8	13.2	0.63	72	1.4	1.2
10	20.0	1.00	87	1.5	1.3
12	33.1	0.98	186	1.6	1.8
14	33.1	1.30	186	1.9	2.3
16	42.2	1.90	272	1.9	3.0
18	29.1	1.36	295	2.5	3.0

* Mean score on a 4 point scale of
1=Typical and fresh, 2=Blank (incipient rancidity);
3=Just noticeable rancidity; 4=Clearly noticeable rancidity.

Results and Discussion

Sensory evaluation data were analysed for mean score for each day's testing. Table 2 gives the result. The chemical analysis were done on the same day. Table 3 gives these results.

Since the experimental samples were stored in air-tight tins without opening till the withdrawal, temperature was the only effective variable. None of the samples except the samples at 37°C reached even the 'just rancid' stage.

Commercial samples which are subjected to constant disturbances unlike laboratory samples, were next analysed for sensory quality and chemical tests. Table 4 gives the mean score of these samples.

The sensory analyses for odour and flavour were treated as two separate attributes and the regression analysis was carried out individually on the three chemical tests: anisidine value, peroxide value and Kreis test, under each condition of storage over the storage period.

Anisidine value and sensory score: Anisidine value has been shown to correlate well with oxidiation and flavour in oils[18,19] fats and in dried emulsions[20]. However, there have also been contradictory observations by other workers especially with oils[21]. The present study corroborates that no significant relationship exists between anisidine value and either odour or flavour at early stages of rancidity under the three storage temperature conditions. Similar findings were noticed with commercial samples also.

Peroxide value and sensory score: Peroxide value, the measure of initial and primary products of lipid oxidation has frequently been used in predicting quality of edible oils like soya bean[4,5], safflower, corn oil and other fats[6], and even for improving the correlation coefficients in a vegetable oil[8]. However, observations have also been made that high peroxide usually means poor flavour score, but low peroxide value does not necessarily mean high flavour score[5], indicating low correlations. In this study the relationship between PV and odour as well as flavour was found to be significant.

TABLE 4. ANALYSIS OF CHEMICAL AND SENSORY PARAMETERS OF COMMERCIAL SAMPLES OF GROUNDNUT OIL

Sample no.	Peroxide value	Kreis value	Anisidine value	Sensory score*	
				Odour	Flavour
1	3.2	0.31	18.3	1.5	1.6
2	2.3	0.21	11.9	1.9	1.8
3	2.6	0.29	13.8	1.3	1.7
4	3.5	0.31	10.6	1.6	1.5
5	3.0	0.30	8.4	1.8	1.5
6	3.4	0.35	12.8	1.5	1.1
7	24.2	1.08	143.0	2.9	2.7
8	6.3	0.27	24.0	1.5	1.4
9	5.7	0.26	26.0	1.5	1.5
10	15.4	0.79	95.0	2.4	2.2
11	3.2	0.31	10.0	1.5	2.0
12	4.7	0.31	21.0	1.7	1.1

*Scale as in Table 2.

The actual regressions with experimental samples are presented in Fig 1 *a* for odour and Fig 2 *a* for flavour along with respective predictive equations. The regression reveals that even at a peroxide value of 30, the odour and flavour will be just beyond 'incipient rancidity' stage.

When the data from commercial samples were considered along with those of experimental samples the regressions were found to be heterogeneous as analysed by Steel and Torris method[22] and the F value was significant at 5 per cent indicating significant non-homogeneity. However, with the exclusion of the regression for the samples stored at 37°C in this total analysis the other regressions became homogeneous. Peroxide value is, as often noted, non-specific because it analyses the transitory hydroperoxides, and this is confirmed by the non-homogeneity of the regressions at elevated temperature of 37°C. But since the regressions of other conditions are homogeneous, it is possible to give a functional relationship between peroxide value and sensorily perceived odour and flavour. The total regressions including the commercial samples but excluding the 37°C samples indicate that at a PV of around 20, 'incipient rancidity' is predicted while at PV 50 the rancidity will be 'just noticeable' both in odour and flavour. The actual regressions with all these samples are represented in Fig 1 *b* for odour and Fig 2 *b* for flavour along with their respective predictive equations.

From these results it can be concluded that though related to sensory properties of odour and flavour, a value of 10 for peroxide value does not represent the cut off point for non-acceptance for groundnut oil under commercial conditions. However, the maximum limit could not be fixed as the odour and flavour scores in these studies did not go beyond 'just noticeable rancidity' and extrapolation is not valid until it is confirmed that the slope will not change in sensory parameter.

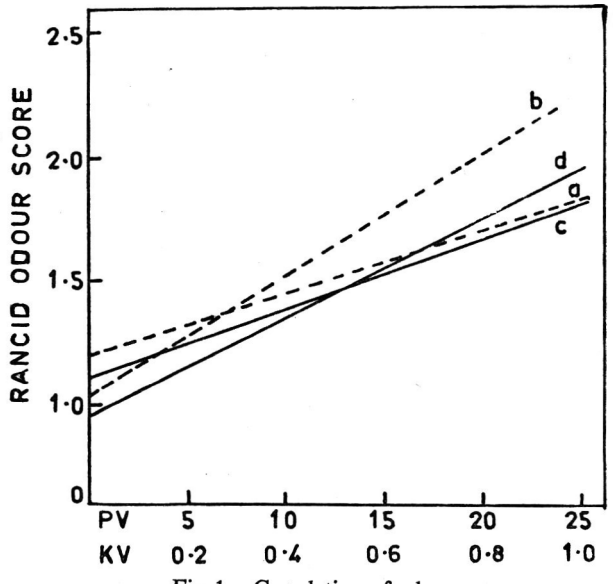

Fig 1. Correlation of odour

PV (.....) of a=experimental sample, (Y=1.2343 + 0.024 x), b=total sample (Y=1.0498 + 0.0484 x) and with Kreis value (KV) (———) of c=experimental sample (Y=1.1065+0.7129 x) and d=total sample (Y=0.9748 + 0.9702 x) where Y is the estimated value of PV or KV and x is the predicted score for odour; sacle for rancid odour 1=typical groundnut oil, fresh, not rancid 2=incipient rancidity, bland, 3=slight ranciuity and 4=clear rancidity.

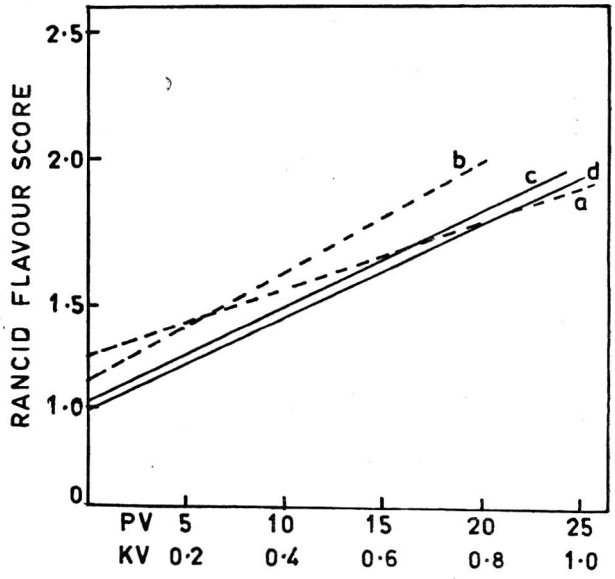

Fig 2. Correlation of flavour

PV (.....) of a=experimental sample, (Y=1.1983+0.0279 x) b=total sample (Y=1.1107+0.0435 x) and the Kreis value (KV) (———) of c=experimental sample (Y=1.0575+0.8931 x) and d=total sample (Y=0.9340+1.0715 x), where Y is the estimated value of PV or KV and x is the predicted score for flavour. Scale for rancid flavour 1=typical groundnut oil, fresh, not rancid, 2=incipient rancidity, bland 3=slight rancidity and 4=clear rancidity

Kreis test and sensory score: Kreis test is a simple rapid test used to assess oxidation of fats and oils by the measurement of red colour developed when phloroglucinol is added to the fat in acid solution.[15] However, many workers have expressed doubts regarding its validity as the colour does not necessarily parallel the degree of rancidity. In the present study, it was included as one of the chemical tests because of its use by public analysts and hence to check its validity.

The test shows significant relationship, in experimental and commercial samples both with odour and flavour. The regression over the storage conditions were not found significantly different in slope as well as the constant as shown by the regression analysis and hence could be represented by a signle linear regression.

The actual regression with experimental samples are presented in Fig 1*c* for odour and Fig 2*c* for flavour along with respective predictive equations. In the study of homogeneity of regression of commercial samples along with test samples, Kreis test values were found to be homogeneous both for odour and flavour. Hence a total regression was worked out and is presented in Fig 1*d* for odour and Fig 2*d* for flavour along with their respective predictive correlations. The regression indicates that a low Kreis value can distinctly predict superior quality of oil while a high Kreis value will indicate deterioration of the quality. The panel has been found equally accurate and consistent in the analysis of both odour and flavour. It will be worth considering odour evaluation alone here.

Since the slope is more in correlation of Kreis value and sensory scores than in peroxide value and sensory score and also the commercial and the test sample reflect similar relationship in Kreis test, it is more valid to use Kreis test for evaluating onset of rancidity in early stages. However, the predictability of the definite Kreis value at the stage of sensorily noticeable rancidity will have to be established by further experimentation with a larger sample and cannot be predicted from this study through extrapolation.

Though both peroxide value and Kreis test have shown good correlation, Kreis test seems to be more promising in predicting early stages of rancidity.

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Natural Plant Enzyme Inhibitors, Action of Seed Proteinase Inhibitors on Ten Different Pancreatic Preparations

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Inhibitions of total proteolytic (casein hydrolysis), tryptic (benzoyl DL-arginine p-nitroanilide hydrolysis) and chymotryptic (acetyltyrosine ethyl ester hydrolysis) activities in ten different pancreatic preparations by aqueous extracts of winged bean (*Psophocarpus tetragonalobus*), Indian red wood (*Adenanthera pavonia*) seed, jack fruit (*Artocarpus heterophyllus*) seed, sword bean (*Canavalia ensiformis*) and sesbania (*Sesbania aegptica*) seed were tested. Total proteolytic and tryptic activities of rabbit pancreas were maximally inhibited by the seed inhibitors compared to other pancreatic systems. Dog chymotrypsin was found to be highly susceptible to the seed inhibitors whereas rat chymotrypsin was resistant to inhibition by the factors other than that from winged bean. Sword bean extract had no inhibitory action on the three hydrolytic activities of the human pancreatic preparation and the sesbania inhibitor exhibited minimal action on the human total proteolytic and tryptic activities. Even though horse and cat trypsins were powerfully inhibited by extracts of jack fruit seed and red wood seed respectively, this was not reflected in inhibition of total proteolytic activity. The reasons for the variations are discussed.

Although many plant proteinase inhibitors are known to act on similar enzymes from more than one animal source the emphasis in these studies has been on comparison of the action of inhibitors on human pancreatic enzymes with bovine[1-4], rat[5] or dog[6] enyzmes. In nutritional studies to assess the effect of proteinase inhibitors, smaller experimental animals are employed whereas in *in vitro* investigations, purified bovine pancreatic enzymes are used. This gap could be bridged by comparing the action of inhibitors against different animal pancreatic proteinases. Such studies will also help in choosing an animal system close to the human for nutritional studies based on similarities in inhibitory patterns. Further, studies with crude pancreatic extracts and the use of extracts of plant materials rather than purified inhibitors, will reflect more closely the situation *in vivo* and the information obtained will be more meaningful from the nutritional view point. In view of this, we have undertaken a study on the comparative action of inhibitors from five plant seeds on ten different pancreatic systems with reference to the diminition of total proteolytic, tryptic and chymotryptic activities.

Materials and Methods

The following seeds were procured locally: Indian red wood seed (*Adenanthera pavonia*), winged bean (*Psophocarpus tetragonalobus*), sword bean (*Canavalia ensiformis*) jack fruit (*Artocarpus heterophyllus*) seed and sesbania (*Sesbania aegptica*) seed. Pancreatic acetone powders from rabbit, rat, cat, dog, horse, pig, guinea pig and chicken, benzoyl DL-arginine *p*-nitroanilide (BAPNA) and acetyl-tyrosine ethyl ester (ATEE) were got from Sigma Chemical Company, St. Louis, MO, U.S.A. Preparation of human and bovine pancreatic acetone powders and the activation of the proenzymes in the acetone powders were described earlier[2]. Other reagents were of analytical grade chemicals.

Caseinolytic activities of activated pancreatic extracts were determined by the procedure of Sumathi and Pattabiraman[7]. In routine experiments (20 min incubation, 37°C, pH 7.6), 70 μg (protein) of human, 45 μg of bovine, 70 μg of rabbit, 60 μg of rat, 50 μg of cat, 35 μg of dog, 60 μg of horse, 70 μg of pig, 50 μg of guinea pig and 120 μg of chicken pancreatic preparations were used to get an optical density value of 0.6 (λ540). In the determination of trypsin amidolytic activity[8] under the assay conditions (15 min incubation, 37°C, pH 7.6), 180 μg (protein) of human, 160 μg of bovine, 125 μg of rabbit, 120 μg of rat, 130 μg of cat, 40 μg of dog, 140μg of horse, 180 μg of pig, 125 μg of guinea pig and 260 μg of chicken pancreatic preparations gave an O.D. value of 0.6 (λ410). Chymotrypsin esterase activity was determined with ATEE[9]. Under the conditions (10 min incubation, 37°C, pH 7.6) of

estimation, 15 μg (protein) of human, 6 μg of bovine, 28 μg of rabbit, 30 μg of rat, 18 μg of cat, 20 μg of dog, 90 μg of horse, 16 μg of pig, 20 μg of guinea pig and 20 μg of chicken pancreatic preparations produced acetyl tyrosine equivalent to 0.6 O.D. (λ540).

Seed extracts for inhibition studies were prepared as follows. Finely powdered seed (2 g) was homogenized with 20 ml of 0.1 M sodium phosphate buffer pH 7.6, stirred for 30 min and centrifuged at 10,000 $\times$ g for 20 min at room temperature. The supernatant was dialyzed overnight against 50 volumes of the same buffer and was used as the source of inhibitor. The proteinase inhibitory activity was assessed by the decrease in hydrolytic activity on inclusion of the seed extracts in the assay systems. The seed extracts themselves did not have measurable hydrolytic activities under the assay conditions. One unit of inhibitory activity is the amount that suppressed the hydrolytic activity (caseinolytic or amidolytic activity as the case may be) by one absorbance unit. For anti-chymotryptic activity, one unit was equivalent to the amount that suppressed esterolytic activity by ten absorbance units. The inhibitory units were quantitated based on the magnitude of inhibition in the linear range. For this purpose, widely varying volumes of the plant extracts in duplicate were included in the assay system and the inhibitory profiles were assessed. Generally, caseinolytic inhibition was linear upto 30-60 per cent and tryptic inhibition and chymotryptic inhibition upto 60-90 per cent for different seed extracts.

Protein in the seed extracts and pancretatic preparations was determined by the method of Lowry *et al.*[10] using bovine serum albumin as standard.

Results and Discussion

The data on inhibition of caseinolytic activity in different pancreatic preparations by the seed extracts are shown in Table 1. Jack fruit seed, red wood seed and winged bean diminished the proteolytic activity of the rabbit pancreas maximally. While the jack fruit inhibitor acted on the chicken preparation also effectively, red wood seed inhibitor had preferential action on rat preparation. Sword bean and sesbania seed inhibitors had feeble action on the human preparation. Comparison of the amount of the inhibitors required to cause 50 per cent inhibition of the caseinolytic activity and correlation of this information with data in Table 1 indicate that rabbit system is most susceptible to inhibition by all the plant seed inhibitors followed by dog. The human pancreatic extract on the same count appears to be minimally affected.

Relative inhibition of tryptic activity in different pancreatic extracts (Table 2) shows that rabbit trypsin is highly inhibited by all the five seed preparations. Equine trypsin was found to be inhibited slightly more powerfully than the rabbit enzyme by jack fruit seed extract and sesbania seed extract. While sword bean extract had no action on human trypsin, sesbania inhibitor was least effective on the human enzyme correlating well with the data on caseinolytic inhibition.

Rabbit chymotrypsin was not highly susceptible (Table 3) to all the seed extracts. Dog chymotrypsin was powerfully inactivated by the inhibitors in jack fruit seed, red wood seed, and winged bean. Human chymotrypsin was most powerfully inhibited by red wood seed extract, whereas horse, chicken and bovine chymo-

TABLE 1. RELATIVE INHIBITION* OF CASEINOLYTIC ACTIVITY IN PANCREATIC EXTRACTS BY SEED PREPARATIONS

Pancreas	Jack fruit seed	Red wood seed	Winged bean	Sword bean	Sesbania seed
Rabbit	109.0	64.6	52.9	1.88	5.83
Chicken	101.0	38.2	10.4	0.15	1.67
Rat	32.6	63.0	11.8	0.05	0.68
Dog	54.3	44.4	23.6	2.80	5.83
Man	22.6	21.8	15.3	0.00	0.08
Guinea pig	38.0	29.4	11.1	0.23	1.56
Horse	48.9	52.9	19.4	0.13	1.83
Ox	38.8	18.2	10.3	0.60	2.29
Pig	48.9	32.9	25.0	0.40	2.08
Cat	65.2	29.4	14.6	0.18	3.33

*Inhibitory units/mg protein.

TABLE 2. RELATIVE INHIBITION* OF TRYPTIC ACTIVITY IN PANCREATIC EXTRACTS BY SEED PREPARATIONS

Pancreas	Jack fruit seed	Red wood seed	Winged bean	Sword bean	Sesbania seed
Rabbit	101.0	279.0	41.70	3.00	16.70
Horse	120.0	44.1	22.20	0.90	20.00
Man	46.2	42.0	8.33	0.00	0.83
Guinea pig	32.8	35.2	8.68	0.70	3.73
Cat	65.2	103.0	27.80	2.75	12.50
Dog	59.8	51.9	15.30	2.50	10.80
Pig	54.3	58.8	12.50	1.00	8.33
Chicken	59.8	22.1	5.56	1.00	8.33
Rat	48.9	35.2	9.72	1.75	10.00
Ox	43.8	29.0	6.94	1.20	8.75

*Inhibitory units/mg of protein.

TABLE 3. RELATIVE INHIBITION* OF CHYMOTRYPTIC ACTIVITY IN PANCREATIC EXTRACTS BY SEED PREPARATIONS

Pancreas	Jack fruit seed	Red wood seed	Winged bean	Sword bean	Sesbania seed
Dog	20.7	47.10	27.80	0.850	1.00
Rabbit	6.52	20.50	26.40	1.400	0.42
Man	15.20	54.10	4.86	0.000	0.06
Horse	2.03	5.19	3.06	0.025	3.17
Chicken	10.30	29.40	1.39	0.100	2.83
Ox	14.10	41.60	4.86	0.150	2.50
Rat	1.90	1.03	6.25	0.002	0.01
Pig	9.24	22.10	19.40	0.004	0.64
Guinea pig	7.61	1.32	1.11	0.400	0.02
Cat	7.61	17.60	16.70	0.700	0.40

*Inhibitory units/mg of protein.

TABLE 4. RELATIVE RATIOS OF TRYPTIC/CHYMOTRYPTIC INHIBITIONS IN PANCREATIC EXTRACTS BY SEED INHIBITORS

Pancreas	Jack fruit seed	Red wood seed	Winged bean	Sword bean	Sesbania seed
Rat	25.70	34.20	1.55	875.00	1000.00
Horse	59.10	8.50	7.25	32.00	6.31
Rabbit	15.50	13.60	1.74	2.14	39.80
Cat	8.57	5.85	1.66	3.93	31.60
Pig	5.88	2.66	0.64	250.00	13.00
Chicken	5.80	0.75	2.00	10.00	2.94
Guinea pig	4.31	26.70	7.82	1.75	15.70
Man	3.04	0.78	1.71	—	13.80
Dog	2.89	1.10	0.55	2.94	10.80
Ox	3.11	0.70	1.43	8.00	3.50
Crystalline bovine enzymes	2.76	0.69	1.36	8.00	4.50

trypsins were highly susceptible to sesbania seed inhibitor. Jack fruit seed, red wood seed, sesbania seed and sword bean inhibitors exhibited minimal action on rat chymotrypsin. Human chymotrypsin was not affected by sword bean inhibitor.

The relative ratios of tryptic inhibition (BAPNA as substrate) to chymotryptic inhibition (ATEE as substrate) in the different pancreatic extracts by the seed inhibitors are shown in Table 4. For comparison, the ratios obtained with crystalline bovine enzymes are also shown. These ratios may be considered as indices of the relative susceptibilities of trypsin and chymotrypsin to an inhibitor. The ratios for the same seed preparation varied over a wide range in regard to the different pancreatic extracts. The values indicate that no single plant inhibitor can be classified as a trypsin inhibitor or trypsin/chymotrypsin inhibitor in a conventional sense, since the patterns of inhibition appear to depend more on the animal system than on the inhibitor. Among the five plant seeds studied red wood seed has been investigated in detail for its inhibitory action[11]. It has been shown that this seed contains a single inhibitor acting both on bovine trypsin and chymotrypsin effectively. Data in Table 4 indicate that while human, bovine, chicken and dog trypsins and chymotrypsins are affected to comparable degrees by red wood seed extract, rat and guinea pig trypsins were inactivated more powerfully than the corresponding chymotrypsins.

In regard to inhibition of overall proteolytic activity and tryptic activity the rabbit system was found to be maximally affected by the plant inhibitors. This suggests that tryptic inhibition might be the crucial factor in determining the diminition in total proteolysis. However, a closer scrutiny of the data fails to support this view. Even though equine trypsin was affected by jack fruit seed as effectively as the rabbit enzyme, the inhibition of caseinolytic activity was only half as effective with the equine pancreas. This does not appear to be compensated by increased inhibition of equine chymotrypsin by jack fruit seed. Conversely, while the caseinolytic activities of rabbit and rat pancreatic preparations are equally affected by red wood seed inhibitor, rabbit trypsin was affected by an order of magnitude more powerfully than the rat enzyme. These variations could be due to differences in the relative proportion of trypsin, chymotrypsin, elastase and carboxypeptidases and their contribution in individual pancreatic extracts to overall proteolytic activity. Krogdahl and Holm[5] indicated that 50 per cent of the total proteolytic activity in dog and human pancreatic secretions is contributed by trypsin and chymotrypsin and the remaining activity was shared equally by elastase and carboxypeptidases. On the other hand, Mallory and Travis[12] concluded that one third each of total proteolytic activity in human system is contributed by trypsin and chymotrypsin and about 25 per cent by elastase. Prabhu and Pattabiraman[2] observed that in bovine pancreatic preparation, carboxypeptidase and elastase accounted for only 5 per cent and 15 per cent of the total activity respectively. An additional factor will be the variations in the magnitude of inhibition of the endopeptidases depending on the substrates used. Earlier reports on the weak action of soya bean inhibitors on human trypsin[4,12] was attributed[3] to the different substrates used. These data

suggest that extrapolation of information obtained on the inhibition of tryptic and chymotryptic activity with synthetic substrates need not reflect the inhibition profiles obtained with protein substrates.

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Effect of Low Dietary Protein on the Mammalian Toxicity of Technical X-Factor*

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The effect of low protein diet on the acute and subacute toxicity of technical X-factor to rats was investigated. In acute study, in female rats fed low protein (LP) and Normo protein (NP) diet for 45 days the oral LD_{50} were 0.78 and 5.11 g/kg body weight respectively. In subacute study, male weanling rats were fed X-factor at 25 and 1500 ppm in either LP or NP diet for 90 days. Rats of only LP group developed signs of X-factor poisoning and a 33.3% mortality occurred at 1500 ppm. While a high degree of hepatic hypertrophy and marked testicular atrophy were evident in LP group rats at 1500 ppm, significant changes in the levels of both serum and liver enzymes (transaminases and G-6-Pase) were observed among both dietary groups.

That the composition of the diet is an important factor in affecting the toxicity of pesticides is well demonstrated[1-3]. Protein has been the most studied nutrient as it is known to affect the principal enzyme systems viz., mixed function oxygenases (MFO) which are primarily involved in the detoxification of pesticides and other xenobiotics[4].

*a by-product obtained during the separation of lindane from technical BHC.

Boyd[3] showed that the toxicity of various pesticides increased in rats fed diets containing no or low protein (0-5 per cent) compared to those fed high (26 per cent) protein diets. Subsequent studies have clearly proved that a decrease in either quality or quantity of protein decrease MFO activity which generally leads to increased toxicity of xenobiotics, as their metabolism is reduced[5].

Technical X-factor (a by-product obtained during the separation of lindane from technical benzene hexachloride) showed very low acute oral toxicity to albino rats[6]. Further, dietary X-factor also showed relatively low subacute toxicity compared to its parent material technical BHC[7]. The present investigation was aimed at studying the effect of low dietary protein (poor rice diet, 7 per cent protein) on the acute and subacute toxicity of technical X-factor.

Materials and Methods

Acute study: Seventy female weanling rats (*Rattus norvegicus*, Wistar-CFT strain, 40-45 g body weight) were randomly divided into two groups. Group A rats were assigned the Normo protein diet (17 per cent protein, NP) and Group B rats, the low protein diet (7 per cent protein, LP) (Table 1). Caged individually, they were maintained on their respective diets (*ad libitum*) and water for 45 days, at the end of which they were statistically grouped by randomized design into groups of six each.

All the animals were partially fed prior to the intragastric administration of the insecticide. Technical X-factor was finely ground in a pulveriser and a solution/suspension of suitable concentration in groundnut oil was prepared. Group A rats were administered at the dosages of 2, 3, 4 and 6.75 g/kg body weight. While group B rats at the dosages of 0.4, 0.6, 0.89 and 1.33 g/kg body weight. Separate controls were maintained for both dietary groups. Treated animals were observed for the onset of any clinical symptoms and mortality. Maintaining the records on weekly body weights, all survivors were autopsied after chloroform anaesthesia at the end of 3 weeks. Recording the weights of vital organs, a portion of each was fixed in 10 per cent neutral formalin and processed for histological examination. The mortality data were analysed by probit regression analysis to arrive at the LD_{50} and LD_{90} values.

Subacute study: Seventy two, 21 day old, male weanling rats (50-55g) were allotted to two dietary groups and statistically grouped by randomized design. Desired concentration of technical X-factor was prepared in groundnut oil and incorporated into each portion of the diet (Table 1) to achieve dietary levels of 25 and 1500 ppm. Caged individually, they were conditioned for first two days by feeding on their respective pesticide-free diet. Group A rats received X-factor incorporated NP diet at 0, 25 and 1500 ppm, while group B rats received similar levels in LP diet. Rats were fed *ad libitum* and had free access to water. Daily food intake, weekly body weights were recorded and animals observed for any noticeable symptoms. At the end of 90 days, survivors were autopsied after light chloroform anaesthesia and the vital organs excised. Recording their fresh weights, portions of each organ were fixed in 10 per cent neutral formalin and processed for histological examination.

For biochemical analysis, a 10 per cent homogenate of liver was prepared in 0.25 M sucrose, centrifuged at 4,000 rpm for 15 min and the supernatant used for enzyme assay. For sera, blood was drawn directly from the heart, allowed to clot at room temperature, centrifuged and kept frozen until use. The following enzymes were assayed in serum and liver: glutamate-oxaloacetate transaminase, (GOT, EC. 2.6.1.1)[8], glutamate-pyruvic transaminase, (GPT, EC. 2.6.1.2)[8], lactate dehydrogenase (LDH, EC. 1.1.1.27)[9], alkaline phosphatase, (ALP, EC. 3.1.3.1)[10] and glucose-6-phosphatase (G-6-Pase, EC. 3.1.3.9)[11].

TABLE 1. COMPOSITION OF LOW PROTEIN AND NORMO PROTEIN DIETS

Low (7%) protein	(g/100g)	Normo (17%) protein	(g/100g)
Rice flour	79.00	Corn starch	69.40
Tur dhal	5.00	Casein	17.60
Groundnut oil	5.00	Salt mixture	2.00
Common salt	0.30	Groundnut oil	9.00
Casein	0.32	Vitaminized starch	1.00
Vegetable powder+	10.40	Vitaminized oil*	1.00

+Potato, brinjal and amaranthus powders were mixed in the proportion of 2:2:6.4 % respectively.

*Shark liver oil supplied the daily requirements of A, C and D vitamins.

Results

Acute study: At the end of six weeks feeding on NP and LP diets, the mean body weights of rats were, NP-133g; LP-80 g. On X-factor administration, typical signs of CNS poisoning were observed among both dietary groups within 24 hr. Predominant symptoms were: initial dullness, lacrimation, incoordination of hind limbs, paralysis and convulsions preceding death. But for a slight decrease in food intake during first three days

TABLE 2. ACUTE TOXICITY OF TECHNICAL X-FACTOR TO FEMALE ALBINO RATS

Dosage (mg/kg b.w.)	Mortality (%)	LD_{50} (mg/kg b.w.)	LD_{90} (mg/kg b.w.)
Normo protein (17%)			
2000	0		
3000	16.6	5111	6584
4500	33.3	(3761-6419)	
6750	100.0		
Low protein (7%)			
400	16.6		
600	16.6	784	1584
890	66.6	(516-1305)	
1330	83.3		

Values in parenthesis denote lower and upper confidence limits.

of post treatment, terminally no significant differences were noted in the weight gain of treated survivors compared to their controls.

Among NP group, significant increase in the relative liver weights and marginal increase in kidney and adrenal weights were observed, though only liver weights increased in LP group. Major histological changes evident were: marginal cellular hypertrophy, mild cellular infiltration and cytoplasmic vacuolisation in liver among both dietary groups. The statistically computed LD_{50} and LD_{90} values are indicated in Table 2.

Symptoms, food intake and body weights in subacute study: Rats fed 1500 ppm X-factor in LP diet developed overt signs of toxicity within 3 weeks and 4 rats died before 11 weeks. However, NP group rats showed no adverse symptoms. A 20 per cent reduction was noted in the food intake of rats of LP control group compared to NP controls. A significant reduction in food intake was evident throughout the experimental period at 1500 ppm among both dietary groups. The mean total pesticide intake in mg/rat/90 days was: LP_{25}-23mg; LP_{1500}-1020 mg; NP_{25}-32 mg; and NP_{1500}-1365 mg. Terminally the weight gain in LP controls was 50 per cent less than that of NP controls. At 1500 ppm, a significant reduction in absolute body weight was evident among both dietary groups (Table 3). The cumulative weight gain of rats of both dietary groups is presented in Fig 1 and 2.

Organ weights and histological observations: A general decrease was evident in the absolute organ weights of LP controls compared to NP controls. At 1500 ppm a significant increase was noted in the weights of liver, kidney, and testis among NP group. However, marked decrease in both absolute and relative weights of testis was noted at 1500 ppm in the LP group (Table 3) Microscopic examination of liver revealed mild to moderate hypertrophy with marked cytoplasmic vacuolisation among both dietary groups, being more pronounced in the LP_{1500} group (Fig 3). Other notable changes were: mild vacuolisation in epithelial cells of kidney tubules and marked vacuolisation in cortical cells of adrenals. Though the testis of NP rats at 1500 ppm showed normal histoarchitecture; those of LP group showed reduced seminiferous tubule diameter, reduced germinal epithelium, and varying degree of spermatogenetic arrest (Fig 4).

TABLE 3. EFFECT OF DIETARY X-FACTOR ON ORGAN WEIGHTS OF MALE RATS

Group*	Terminal body wt. (g)	Relative organ wt. (g/100g b.w.) Liver	Kidney	Adrenals	Testis
		Normo (17%) Protein			
Control	306.8±5.3	3.77 ±0.05	0.64 ±0.01	0.013±0.001	0.92 ±0.02
25 ppm	301.6±5.5	3.59 ±0.06	0.63 ±0.01	0.013±0.001	0.99 ±0.03
1500 ppm	245.1±6.2	6.01^c±0.23	0.91^c±0.05	0.018±0.001	1.16^c ±0.05
		Low (7%) Protein			
Control	154.0±3.2	3.40 ±0.07	0.68 ±0.01	0.023±0.006	1.67 ±0.05
25 ppm	136.4±5.8	3.77 ±0.09	0.69 ±0.02	0.024±0.02	1.61 ±0.04
1500 ppm	102.4±3.1	7.24^c±0.23	0.78^c±0.03	0.021±0.002	0.72^c ±0.15

*Each group comprised 12 animals excepting LP_{1500} which comprised only 8 animals. Students 't' test c=p<0.001.

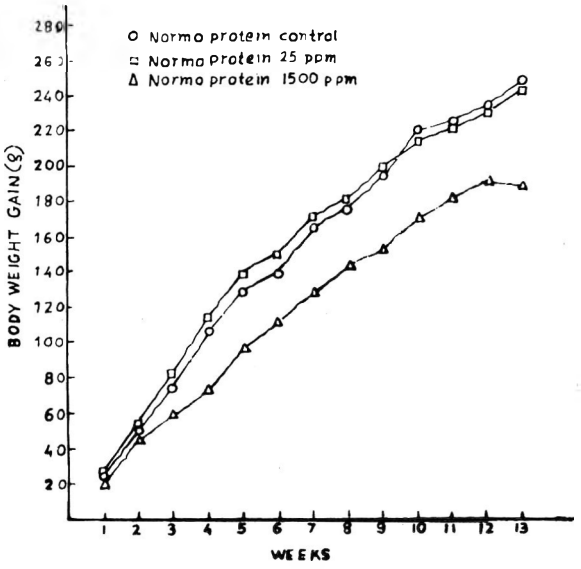

Fig 1. Cumulative body weight gain of growing male rats fed X-factor in Normo protein diet.

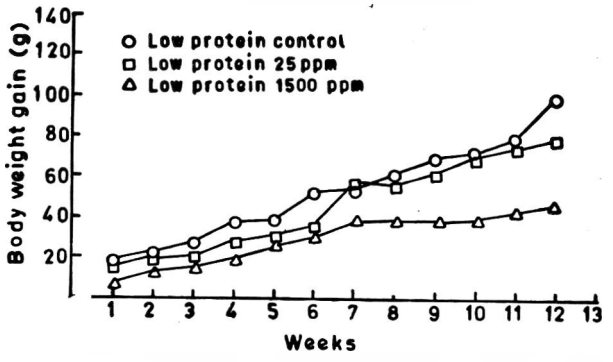

Fig 2. Cumulative body weight gain of growing male rats fed X-factor in low protein diet.

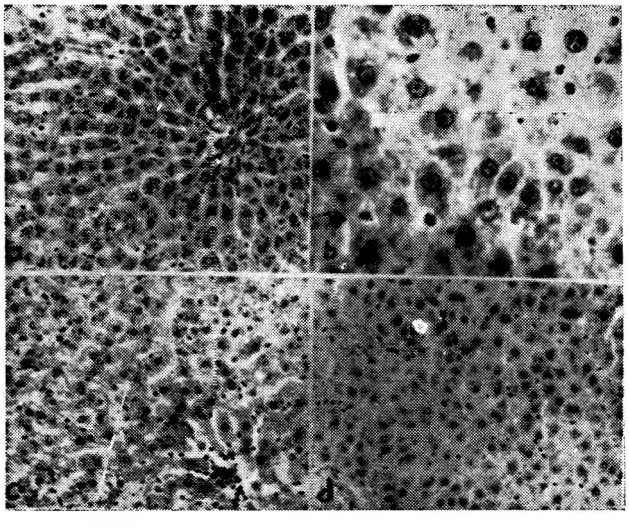

Fig 3. Liver sections of rats fed Normo protein (*a*) and low protein (*c*) diets—note normal histology × 100; Rats fed x -factor (1500 ppm) in NP diet (*b*) ×250; and LP diet (*d*) ×100–note cellular hypertrophy Haematoxylin and eosin (H & E).

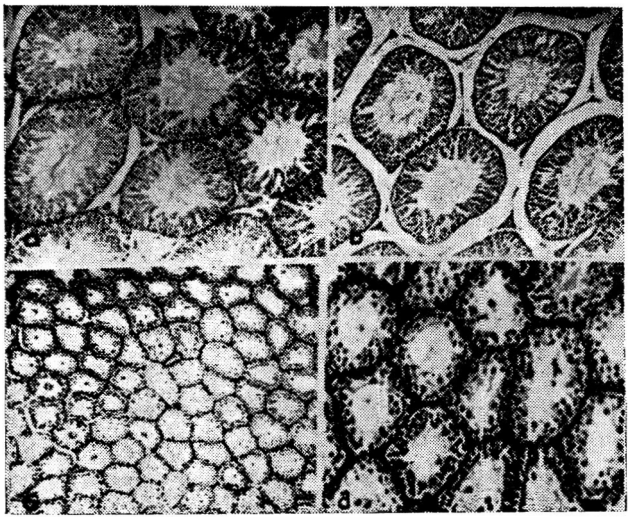

Fig 4. Sections of testis of rats fed NP (*a*) and LP (*b*) diets—note normal histology × 40; Rats fed x-factor (1500 ppm) in LP diet (*c*)—note the shrunken seminiferous tubules and degenerative epithelium, ×40; a portion of 'c' enlarged (*d*)—note spermatogenetic arrest ×100. H & E.

Biochemical analysis: No appreciable changes were observed in the activity of any of liver and serum enzymes measured among either of the dietary groups at 25 ppm. However, in liver, a marginal increase in the level of GPT and a marked increase (3-fold) in G-6-Pase were evident in the NP_{1500} group. A similar trend was observed in the LP_{1500} group. (Table 4). In serum a marginal increase in the activity of both transaminases was noted in the NP_{1500} group (Table 5). While the level of GPT was unaffected among LP_{1500} group animals the activities of GOT and ALP were slightly elevated. Further, only a marginal increase in serum cholesterol was noticed at 1500 ppm in both dietary groups.

Dissusion

In the present study, a synthetic diet with casein as the protein source was formulated and designated as Normo-protein (NP) diet while the low protein (LP) diet was formulated based on the south Indian poor rice diet. In the acute study, the growth of LP fed rats at the end of 45 days was 60 per cent to that of NP fed rats. Earlier Boyd[3] has reported the growth of rats fed a diet containing 1/3 of optimal (9 per cent) to be 45 per cent of that of Normo protein (26 per cent) controls.

Oral administration of X-factor elicited similar responses in rats of both the dietary groups. Symptoms were largely those of CNS depression described elsewhere[6,7]. With doses as low as 600 and 890 mg/kg body weight rats of LP group developed symptoms which were similar to those exhibited by rats of NP group beyond the dosages of 3000 mg/kg body weight. The

TABLE 4. EFFECT OF DIETARY X-FACTOR ON LIVER ENZYMES

Enzyme*	Normo protein			Low protein		
	Control	25 ppm	1500 ppm	Control	25 ppm	1500 ppm
GOT	17. 9±0.60	16. 6±0.60	14. 2±0.90	17. 9±2.40	18. 2±1.30	15. 9±0.70
GPT	68. 7±1.70	67. 2±3.20	88. 9±3.80	60. 3±8.70	72. 3±6.50	78.4^c±0.90
LDH	1.34±0.03	1.33±0.06	0.98±0.05	1.30±0.19	1.48±0.09	1.31±0.01
G-6-Pase	3. 4±0.50	2. 8±0.3	12.9^c±0.70	2. 9±0.30	2. 5±0.40	8.6^c±0.40

Student 't' test: $c=p < 0.001$

*GOT, GPT, LDH and G-6-Pase are in μ moles of oxaloacetate, pyruvate, NADH and P_i mg protein^{-1} hr^{-1} respectively.

Values are mean±S.E. of 5 determinations each

TABLE 5. EFFECT OF DIETARY X-FACTOR ON SERUM PROTEINS, CHOLESTEROL AND ENZYMES

Enzymes (IU/l)	Normo protein			Low protein		
	Control	25 ppm	1500 ppm	Control	25 ppm	1500 ppm
GOT	82.0±6.6	78.0±6.9	109.2^c±2.9	69.5±6.2	62.1±7.5	91.5^c±7.2
GPT	7.4±0.2	8.1±1.3	10.9 ±0.5	9.1±1.1	7.4±0.2	9.8 ±0.6
ALP	8.5±0.6	7.7±0.3	8.2 ±0.9	6.9±1.4	6.9±1.1	8.2^c±0.1
Total protein (mg/ml)	74.7±0.7	73.1±3.1	79.9 ±0.8	72.2±1.0	71.7±0.1	73.1 ±2.1
Total cholesterol (mg%)	81.5±1.2	81.2±1.3	117.5^c±4.9	64.7±4.9	62.5±2.7	84.5^c±2.1

Students 't' test: $c=p<0.001$

Values are mean±S.E. of 5 determinations each.

computed LD_{50} values clearly suggested that decreasing the dietary protein to 7 per cent from 17 per cent protein increased the acute toxicity of technical X-factor by seven-fold. Earlier Boyd[3] studied a series of insecticides and fungicides and showed that their toxicities were increased in rats previously fed a diet containing 0-5 per cent protein compared to those fed 26 per cent dietary protein.

In the subacute study, the feeding of LP diet for 90 days retarded the growth of rats by nearly 50 per cent compared to that of NP fed controls. Only the rats of LP group at 1500 ppm developed signs of X-factor poisoning and mortality suggesting that high dietary protein renders protection to rats. A marked inhibition in the growth of vital organs except testis was evident in LP controls compared to NP controls. However, the relative weights of some organs (Table 3) showed an increase due to a significant decrease in the terminal body weights of rats. These results are in conformity with the earlier reports on the effect of no protein and protein dificient (1/3 of optimal) diet in growing male rats[3]. However no histological alterations were observed in various organs of LP controls compared to NP controls.

With X-factor ingestion at 1500 ppm a marked hepatic hypertrophy was evident among both dietary groups, the degree being higher in LP group. Principal pathological alterations in liver induced by dietary X-factor has been described elsewhere[10]. The incidence of histological lesions were relatively more in LP fed rats. Another pronounced toxic effect at 1500 ppm in LP fed rats was marked atrophy of the testis. Similar atrophy of testis in rats fed dieatary BHC at 1500 ppm was reported in our earlier studies[13]. Though testicular atrophy and degenerative changes in seminiferous eipthelium have been reported in experimental animals administered with various insecticides[14-16], the exact mechanism is not known.

The low protein diet did not bring about any marked alterations in the activity of both liver and serum enzymes as their levels were comparable to the controls of NP diet. However, earlier authors[3,17] have reported marked alterations in hepatic transaminases, aldolase and microsomal hydroxylating enzymes in rats fed no

protein diet. At 1500 ppm alterations of both liver and serum enzymes among the two dietary groups of animals were similar.

These observations suggest that the pesticide elicited relatively more toxic effects in rats fed LP diet than those fed NP diet. Similar results have been reported by Lee *at al.*[1], who demonstrated that the toxic manifestations of dieldrin were more marked in rats fed a 10 per cent casein diet than in those fed 25 per cent casein diet. That this increased toxicity of pesticides in rats fed LP diet may result from decreased detoxification has been suggested by studies in which protein deficiency has been shown to depress hepatic microsomal enzyme activity[4,5].

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ERRATA

Paper entitled "Effect of Parboiling on Hydration and ~~and~~ Sedimentation Characteristics of Cassava (*Manihot esculenta* Crantz) Chips" by K. C. M. Raja and A. G. Mathew, Published in this journal 1986, Vol. 23 No. 1. page 39–41.

On page 39, second column, para 2, the lines; *Plain dried chips:* Fresh slices of 4.0–5.0 mm thickness were dried at 58±2°C for 1 hr in a cross flow dryer. Should be read as

Plain dried chips: Fresh slices of 4.0–5.0 mm thickness were dried at 58±2°C for **10** hr in a cross flow dryer.

Toxicity Studies on Turmeric (*Curcuma longa* L.)–Long Term Toxicity Studies in Albino Rats and Monkeys

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Toxicological studies on turmeric (500 mg/kg body weight) and its alcoholic extract (60 mg/kg body weight) were conducted on albino rats and monkeys for 12 and 9 months respectively. Growth response, food and calorie utilization, organ weights, blood constituents and urine analysis of the animals did not show any deviation from those of respective control animals.

Turmeric (*Curcuma longa* L.) is widely used as a natural food colourant and spice, and as a therapeutic agent in the Indian systems of medicine. The joint FAO/WHO Expert Committee on Food Additives[1] included turmeric in the provisional list and has temporarily recommended the acceptable daily intake (ADI) for turmeric and curcumin as 2.5 and 0.1 mg/kg body weight respectively. A survey conducted in India by the National Nutrition Monitoring Bureau, Hyderabad, revealed that normal intake of turmeric ranges from 0.1 to 3.8 g/day/adult. Many workers have examined the nature of the active principles present in turmeric and their possible mode of action[2-10]. But, no systematic toxicity data have been generated to ensure the safety of turmeric or its fractions. Arora *et al.*[11] have found that petroleum ether extract of turmeric was non-toxic. Ramprasad and Sirsi[12] have shown that sodium curcuminate has very low toxicity. Goodpasture and Arrighi[13] however, reported chromosomal aberrations in mammalian cells in the presence of turmeric under *in vitro* condition. Acute toxicity studies conducted in our laboratory revealed that single dose of turmeric at 2.5 g/kg body weight and its alcoholic extract at 300 mg/kg body weight are not toxic[14]. The data obtained from long term toxicity studies in rats and monkeys are reported here.

Materials and Methods

Turmeric powder containing 2.5 per cent curcumin and its alcoholic extract (oleoresin) were supplied by the Discipline of Plantation Products and Flavour Technology of the Institute. The alcoholic extract essentially contained the oil and the pigment (curcumin) of turmeric.

Animal experiments: The feeding schedule for toxicological evaluation was as per the report of joint FAO/WHO Expert Committee on Food Additives[15].

Studies on rats: Weaned Wistar strain albino rats of both sexes weighing about 34 g were randomly alloted into two groups, each group having ten males and twenty females. The animals were housed in individual cages and diet and water were given *ad libitum*. The first group fed on the basal diet served as control. The second group was fed turmeric at a level of 500 mg/kg body weight. A similar set of two groups of rats were fed the basal diet and the basal diet mixed with alcoholic extract of turmeric at 60 mg/kg body weight level. The composition (in per cent) of the basal diet was; corn starch, 72 of the casein, 15; refined groundnut oil, 10; mineral mixture, 2[16] and vitamin mixture, 1[17]. Turmeric powder was incorporated into the diet as such. Since the alcoholic extract was pasty which could not mix uniformly with the diet, and as it was insoluble in water, it was dissolved in little ethyl alcohol, mixed with the diet and air dried thoroughly to evaporate the alcohol. An equal volume of alcohol was added to the control diet and it was air dried. Feeding was continued for one year.

Studies on monkeys: Monkeys (*Macacus* sp.) were the other species of animals used. The adult males were divided into two groups, each consisting of 4 animals. They were housed in individual cages with free access to water and diet. The diet schedule was as follows: grain powder mixture, 100 g; root vegetables, 120 g; sprouted Bengal gram, 120 g and one banana per monkey per day. One group served as control and the other was fed turmeric (500 mg/kg body weight) embedded in the banana. The experiment was conducted for 9 months.

Observations: Gain in body weight was recorded fortnightly. Urine was collected from rats of each group after 6 months of feeding and at the end of the experiment by placing the animals in metabolism cages, the bottoms of which were fixed with glass funnels having

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

Diet		Average body wt. (g) (mean±S.E.) at indicated intervals (wk)						Body wt. gain in monkeys (kg)[a]
		0	4	8	12	24	52	
Control	M	34. 2±0.90	98.2±3.56	180. 2±3.18	240.8±4.33	271.8±3.70	369.3±12. 7	0.39±0.11
	F	33. 3±0.52	95.8±1.15	137. 4±2.81	157.8±3.54	193.5±1.68	250.3± 8.72	—
Turmeric	M	34. 5±0.91	88.8±4.7	170. 5±3.45**	227.6±5.29	269.4±3.94	371.5±12.52	0.33±0.12
	F	34. 4±0.60	90.0±2.16*	132. 7±2.79	159.1±2.88	201.5±2.42**	250.3± 7.81	—
Control	M	38. 0±0.26	107.4±2.14	178. 3±2.32	214.9±2.07	261.8±2.96	358.3± 9.68	—
	F	38. 8±0.58	103.7±1.08	131. 8±1.76	143.1±1.90	184.2±1.44	212.4± 4.62	—
Alcoholic extract	M	36. 6±0.92	110.4±2.01	172. 4±3.16	204.9±3.25	264.4±4.91	343.8±12.58	—
	F	37. 7±0.86	105.0±0.94	128. 6±1.46	139.5±1.44	181.6±1.22	218.9± 4.15	—

[a]Mean of 4 values
M, male (10 animals); F, female (20 animals)
*Significantly different from control ($P<0.05$); **Significantly different from control ($P<0.01$)

stainless steel discs to separate urine and faeces; 24 hr urine samples were qualitatively analysed for sugar, albumin, ketone bodies, bile acids and bile pigments[18]. They were also subjected to microscopic examination. Haematological analysis was carried out after six months of feeding as well as at the termination of the experiment. In case of monkeys, urine and blood analysis was done only at the termination of the experiment. Diet consumption by rats was recorded for the initial 12 weeks period of the experiment to calculate the 'efficiency of food utilization' (EFU) and the 'efficiency of calorie utilization' (ECU) as described by Oser and Oser[19]. At the termination of the experiment, the animals were anaesthetized, dissected, organs were excised, weighed and preserved in 10 per cent formaldehyde for histological examination. The preserved tissues were washed thoroughly in water, dehydrated using different grades of alcohol, embedded in paraffin wax, sectioned (5μ thick) and fixed on slides. The sections were stained with hematoxylin and eosin and examined under the microscope.

Statistical analysis: Statistical analysis of the data was carried out by Student's 't' test for all the data except for WBC differential count where non-parametric test was applied.

Results and Discussion

The survey conducted by the National Nutrition Monitoring Bureau, Hyderabad, showed that the daily intake of turmeric by an adult is upto 3.8 g. Taking a higher quantity of 5 g/day/adult of average body weight of 70 kg and allowing a seven fold safety margin, 500 mg/kg body weight was fixed as the dose for testing. This is equal to 1/5 of the level used in our previous acute toxicity studies[14]. It is equivalent to 12.5 mg curcumin/kg body weight. On the basis of the curcumin content of turmeric, an equivalent amount of alcoholic extract (60 mg/kg body weight) was also tested.

The increase in body weight of animals belonging to different groups are presented in Table 1. Although there is an initial lag in the weight gain by the rats

TABLE 2. FOOD AND CALORIE UTILIZATION RESPONSES OF RATS FED TURMERIC AND ITS ALCOHOLIC EXTRACT*

Diet		Av. food intake (g)	EFU (g/100g food) Mean±S.E.	ECU (g/100 calories) Mean±S.E.
Control	M	1005.0	20.52±0.37	4.63±0.08
	F	842.0	14.76±0.38	3.33±0.09
Turmeric	M	989.0	19.50±0.46	4.40±0.10
	F	872.0	14.28±0.21	3.23±0.05
Control	M	872.0	20.28±0.11	4.58±0.02
	F	781.6	13.32±0.15	3.01±0.03
Alcoholic extract	M	867.6	19.38±0.71	4.38±0.15
	F	781.3	13.02±0.15	2.94±0.06

*During 12 weeks feeding period.
EFU, Efficiency of Food Utilization.
ECU, Efficiency of Calorie Utilization.
M, male (10 animals); F, female (20 animals),

TABLE 3. HAEMATOLOGICAL PICTURE OF RATS AND MONKEYS FED TURMERIC AND ITS ALCOHOLIC EXTRACT

Group	Feeding period (months)	Hb (g/100 ml)	RBC (10^6/mm^3)	WBC (cells/mm^3 × 10^3)	Mean differential count (%)			
					N	L	M	E
Turmeric (Rats[a])								
Control	6	14.0±0.24	6.9 ±0.18	20.63±0.30	18	77	3	2
Turmeric	6	13.8±0.24	6.8 ±0.18	20.00±0.30	18	78	2	2
Control	12	16.5±0.32	9.1 ±0.11	18.16±0.89	27	68	3	2
Turmeric	12	16.0±0.32	8.8 ±0.11	18.09±0.89	29	64	3	4
Alcoholic extract (Rats[a])								
Control	6	15.1±0.25	8.04±0.26	17.62±1.72	26	68	3	3
Alcoholic extract	6	14.9±0.25	7.9 ±0.26	18.75±1.72	27	65	4	4
Control	12	16.4±0.24	7.6 ±0.24	18.22±1.96	22	69	4	5
Alcoholic extract	12	16.7±0.24	7.8 ±0.24	18.57±1.96	23	67	4	6
Turmeric (Monkeys)								
Control	9	13.6±0.30	5.9 ±0.28	15.28±1.90	39	48	9	4
Turmeric	9	14.1±0.30	5.3 ±0.28	13.68±1.90	26	61	9	4

[a]Values are the means of 7 rats in each sex; N. Neutrophils; L, Lymphocytes; M, Monocytes; E, Eosinophils; Basophils were not detected in any of the samples.

receiving turmeric, there was practically no difference between the control and experimental groups after 12 weeks of feeding. The same trend was noticed in female albino rats receiving alcoholic extract of turmeric. The data on food intake presented in Table 2 indicate that the initial difference in body weight was due to lowered food consumption. EFU and ECU (Table 2) calculated on the basis of diet and calorie consumption did not show any marked deviation in experimental rats as against their control animals. The gain in body weight of monkeys fed turmeric was comparable to controls. Urine analysis of both rats and monkeys did not show any abnormality. In one or two exceptional samples, traces of albumin or sugar were observed which was found to be a normal feature even in control animals. Even the blood picture of the rats and monkeys fed turmeric or its alcoholic extract was found to be normal (Table 3).

The gross morphology of different organs and relative organ weights (g/100 g body weight) of animals receiving

TABLE 4. RELATIVE ORGAN WEIGHTS (G/100G BODY WEIGHT)* OF RATS FED TURMERIC AND ITS ALCOHOLIC EXTRACT

Group		Liver	Kidney	Heart	Brain	Spleen
Control	M	2.41±0.07	0.47±0.02	0.21±0.04	0.41±0.01	0.18±0.01
	F	2.41±0.07	0.47±0.01	0.26±0.02	0.65±0.03	0.18±0.01
Turmeric	M	2.48±0.07	0.48±0.02	0.22±0.03	0.42±0.01	0.19±0.01
	F	2.47±0.07	0.49±0.01	0.28±0.02	0.66±0.05	0.18±0.02
Control	M	2.53±0.14	0.48±0.02	0.23±0.01	0.43±0.03	0.16±0.01
	F	2.52±0.06	0.47±0.01	0.26±0.01	0.71±0.02	0.15±0.01
Alcoholic extract	M	2.52±0.13	0.46±0.02	0.24±0.01	0.46±0.01	0.15±0.01
	F	2.50±0.06	0.48±0.01	0.27±0.01	0.74±0.02	0.15±0.01

M, male; F, female
*Mean±S.E.; n=7.

turmeric or its alcoholic extract were similar to those of the control animals (Table 4). The microscopic examination of different tissues revealed a normal histological picture. Hence, these results lead to the conclusion that turmeric and its alcoholic extract are not toxic even at high levels (200 times more than the temporary ADI for turmeric) used in these experiments. Although the chromosomal aberration in mammalian cell culture treated with turmeric was reported[13], the same effect can not be expected in a complex whole animal system since the animal tissues are not exposed to turmeric alone as it happens in the *in vitro* cell culture system. Interference by various body secretions can not also be ruled out. Recently, Bille *et al.*[20] have reported reduction in weight gain and food conversion efficiency, and some histological changes in kidney and urinary bladder of pigs fed 296 and 1551 mg oleoresin of turmeric/kg body weight/day. This differs from our findings and it may be due to the species difference and much higher doses used by Bille *et al.*[20] If 1551 mg oleoresin is extrapolated to a man of 70 kg, about 380 g turmeric (on the basis of curcumin content at 5 per cent of turmeric) per day is required to cause similar adverse effects. This is 100 times more than the actual turmeric consumption by an adult as revealed by the survey of National Nutrition Monitoring Bureau, Hyderabad, India (3.8 g/adult/day). The practicality of consuming 380 g turmeric/adult/day is to be considered before concluding that turmeric can be toxic.

Acknowledgement

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EFFECT OF GAMMA IRRADIATION ON COOKING TIME AND PHYSICO-CHEMICAL CHARACTERISTICS OF BROAD BEANS (*VICIA FABA*) STARCH

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Gamma irradiated broad beans (*Vicia faba*) showed a reduction in cooking time of about 27, 46, 58 and 63% when irradiated at 1.25, 2.50, 5.00 and 10.00 kGy level respectively. Sensory evaluation tests indicated no changes in broad bean flavour quality by irradiation up to 5 kGy. Rheological properties were adversely affected by irradiation, the maximum viscosity of 880 B.U. for the control got reduced to 530 B.U. after irradiation with 5 kGy. Solubility of starch from irradiated sample was increased with concomitant decrease in swelling power. Irradiation was found to cause textural improvements which can enhance the qualities of broad beans.

Leguminous seeds are very important in the diet of people in many areas of the world[1,2]. One of the reasons for their low consumption is the excessive time required for their preparation[3,4]. In order to upgrade the utilization of dry beans, numerous methods to shorten cooking time were devised[5-9]. Gamma irradiation was found to cause a significant reduction in cooking time and improve the textural quality of legumes[10,11]. The aim of the present investigation was to study the effect of gammairradiation on cooking time of broad bean and on some physico-chemical characteristics of their starch.

Broad bean samples, cultivar 'Cyprus' were obtained from the Za 'afarania Experimental Station. Sun-dried seeds were cleaned and kept frozen (–20°C) in sealed polyethylene bags until used. They were exposed at ambient temperature (25°C) to a cobalt 60 close type source of gamma radiation (Gamma cell-220, Atomic Energy of Canada Ltd.,) having a dose rate of 0.019 kGy/min. The time of exposure was calculated to equal the radiation doses of 1.25, 2.50, 5.00 and 10.00 kGy. The irradiated broad bean samples were cooked using the Central American Standard Method[12]. Lots of 500 g of cleaned beans were cooked in 1.5 l of boiling 2 per cent sodium chloride solution. Unirradiated samples served as control.

A Brabender amylograph (Duisburg, W., Germany) was used to determine the maximum viscosity of broad bean flour. Sample was ground and fractionated with 60 mesh sieve. Lots of 80 g flour samples were used with 460 ml distilled water. The starting temperature was adjusted to 25°C, and increased at a rate of 1.5°C/min. The viscosity of the slurry was recorded as the temperature increased from 25 to 95°C. The maximum viscosity was read in Brabender units (B.U.)

Irradiated and unirradiated broad bean samples (75 g) were suspended in ten times the amount of 0.1 M NaCl and centrifuged at 2000 ×g[10]. The upper protein layer was scraped out and the process was repeated three times. The sediment was then washed four times with toluene (0.1 vol.) to reomve the last traces of proteins followed by freeze drying. Isolated broad bean starch (5 g) was dispersed in 100 ml of water and heated for 30 min in a water bath, maintained at the 70,75,80,85,90 and 95°C with gentle stirring. The suspension was, centrifuged at 3000 ×g and the supernatant dried under vacuum. Solubility was determined by weighing the residual dissolved starch. Starch samples were cooked as described for the solubility experiments and centrifuged. Swelling power was measured by determining the water retention capacity of undissolved starch, after making appropriate corrections for the dissolved starch. The swelling power of starch was calculated as per the following equation.[13]

$$\text{Swelling power (corrected)} = \frac{\text{Wt of sedimented paste} \times 100}{\text{Wt of sample (on dry basis)} \times (100 - \%\ \text{solubles})}$$

As indicated in Fig 1, cooking time progressively decreased with increasing dose of irradiation with about

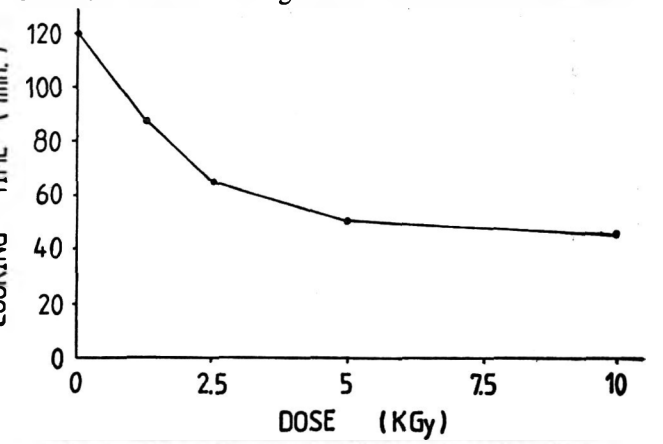

Fig 1. Effect of gamma irradiation on cooking time.

60 per cent reduction at 5 kGy. Sensory evaluation showed that there was no objectionable odd flavour in irradiated (upto 5.00 kGy) cooked samples. Samples irradiated with 10.00 kGy showed slight differences in colour and taste from their control after cooking. A similar observation was also reported for red gram where the time taken for cooking in terms of softening was reduced by 38.5 per cent in irradiated (10.00 kGy) samples compared to the unirradiated one.[10] Since only 5 per cent reduction in cooking time was observed between the 5.00 and 10.00 kGy samples, only the 5.00 kGy treatment was considered in subsequent experiments.

The effect of irradiation (5.00 kGy) on rheological properties of broad beans was measured by using the Brabender amylograph. Fig 2 shows the effect of irradiation on amylograph peaks (maximum viscosity in B.U.); it is seen that the maximum viscosity tended to decrease after irradiation.

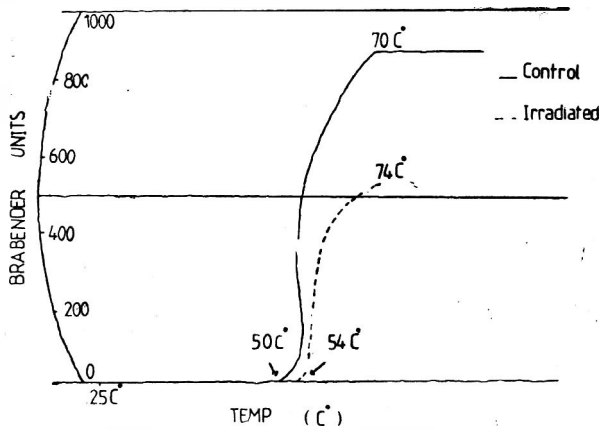

Fig 2. Amylograms of irradiated (5 kGy) and non-irradiated broad bean flour.

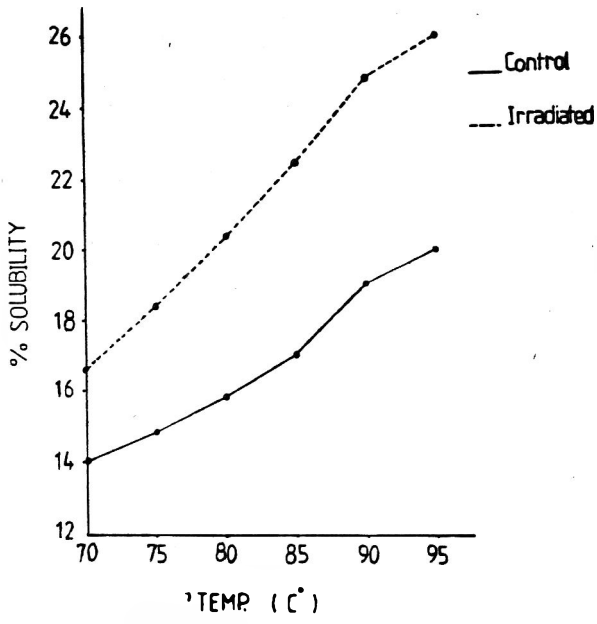

Fig 3. Solubility and swelling power of isolated broad bean starch.

The maximum viscosity was 880 B.U. for the control and became 530 B.U. for the 5.00 kGy irradiated samples. A similar decrease in amylogram units of irradiated red gram starch[10] and wheat starch[14] have been reported. The decrease in viscosity has been attributed to shortening of polysaccharide chain[15], depending upon the radiation dose. This decrease in viscosity of irradiated broad bean flour is probably attributable to the high content of starch of such beans (37.3 per cent)[11].

Changes in solubility and swelling power of starch isolated from control and irradiated (5.00 kGy) broad bean are shown in Fig 3. It can be seen that solubility of starch from irradiated sample increased with concomitant decrease in swelling power.

These results provide adequate evidence that gamma irradiation (5.00 kGy) of broad beans causes alterations in some of their physical properties namely viscosity, mechanical strength, swelling and solubility.

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AVAILABILITY OF L-ASCORBIC ACID IN TANZANIAN BANANA

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Banana is predominantly grown in Kilimanjaro, Mbeya, Kigoma, Arusha, Kagera and Morogoro regions in Tanzania where it is a traditional staple food. The cooking varieties commonly cultivated are 'malindi', 'mzuzu', 'mshale' and 'matoke' and their L-ascorbic acid contents were 7.7, 16.0, 6.3 and 10.3 mg/100g respectively. 'Sukari' and 'malindi' (ripe) are the most common dessert types with ascorbic acid content of 7.9 and 4.4 mg/100g respectively. Boiling and roasting lowered the ascorbic acid content of the unripe 'malindi' by 39.0 and 68.8 per cent respectively.

Banana is one of the most important food crops widely cultivated in Tanzania. Large scale cultivation of banana is predominant in Kilimanjaro, Mbeya, Kigoma, Arusha, Kagera and Morogoro regions. In some of these areas banana is a traditional staple food. Intense cultivation of banana in the country for over a century has led to the establishment of many varieties and cultivars for specific local needs. Apart from the cooking and dessert types, there are varieties specific for brewing and flour making.

Banana and papaya are the only fruits available in all seasons in the country. Where it is a staple, the daily consumption of banana is estimated in the range of 2 to 3 kg per individual. However, with improved situation of food grains, presently the average consumption of banana is estimated to be a little less. In view of its wide consumption, importance is specially given to studies on nutritional value of bananas. β-carotene, and vitamins such as L-ascorbic acid, niacin and inositol are present in moderate quantities while thiamin, riboflavin, pyridoxine, pantothenic acid and folic acid are present in very minor quantities[1]. Ascorbic acid content of banana is generally in the range of 10-30 mg per 100 g.[2] The ascorbic acid content of 22 among 23 different Tanzanian bananas has been found to be below 20 mg per 100 g[3]. Except for tabulating the data on vitamin content in bananas, admittedly little work has been done[4]. The present study describes the effect of traditional cooking of bananas on the ascorbic acid content.

Mature green bananas belonging to varieties 'mshale', 'mzuzu', 'malindi' and 'matoke', and fully ripe bananas of 'sukari' and 'malindi' were locally obtained. Genetic groups of the varieties stated are listed in Table 1. Cooking (boiling) and roasting were traditionally done in an aluminium vessel and on charcoal respectively. Changes in banana temperature were monitored during both cooking and roasting.

Ascorbic acid was determined by the AOAC procedure[5] in all mature green, and ripe bananas as soon as they were obtained. Only the 'malindi' variety was used to estimate the loss of ascorbic acid during cooking by boiling and roasting. Ascorbic acid was estimated every 10 min for 40 min during the cooking process.

The ascorbic acid content of bananas is shown in Table 1. It ranged from 6.3 to 16.0 mg/100 g in cooking varieties, and from 4.4 to 7.9 mg/100 g in dessert types.

TABLE 1. ASCORBIC ACID CONTENT OF GENETIC VARIANTS OF TANZANIAN BANANAS

Banana variety		Genetic group[a]	Maturity stage	Ascorbic[b] acid (mg/100g)
Swahili	Common name			
'Sukari'	Ney Poovan	AB	Ripe	7.9
'Sukari'	Silk	AAB	Ripe	9.3
'Matoke'	Robusta	AAA	Unripe	10.3
'Mshale'	Gros Michael	AAA	Unripe	6.3
'Mzuzu'	Bluggoe	ABB	Unripe	16.0
'Malindi'	Dwarf Cavendish	AAA	Unripe	7.7
'Malindi'	Dwarf Cavendish	AAA	Ripe	4.4

a See reference No. 1.

b Values are the mean of two independent observations.

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There is, as already recorded, a wide variation in the ascorbic acid content in different varieties of banana[3]. The varieties grown in Tanzania have already been described[6]. Unripe, mature fruits of 'malindi' type are used mostly for cooking while the ripe fruits are used as dessert. The decrease in ascorbic acid content during ripening has also been observed by Gomez and Mattill[7].

Since L-ascorbic acid is a water-soluble vitamin and is most easily susceptible to destruction, considerable loss is expected during cooking, especially if it is prolonged[8]. Effect of boiling and roasting on the ascorbic acid content of 'malindi' banana is represented in Fig 1.

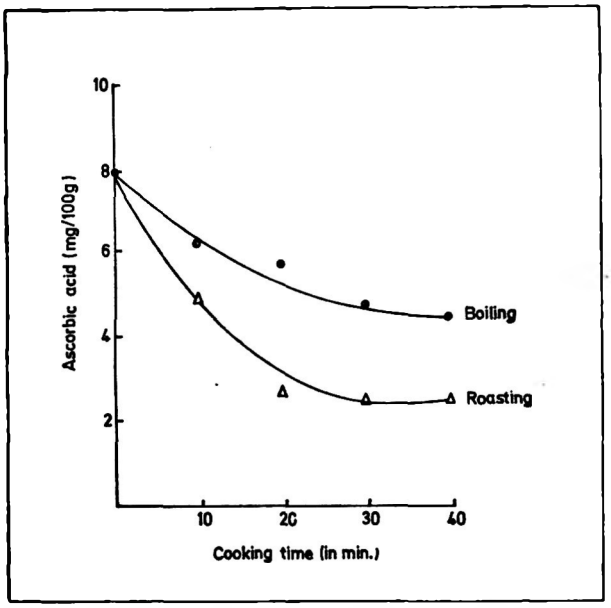

Fig 1. Effect of boiling and roasting on the ascorbic acid content of 'malindi' banana.

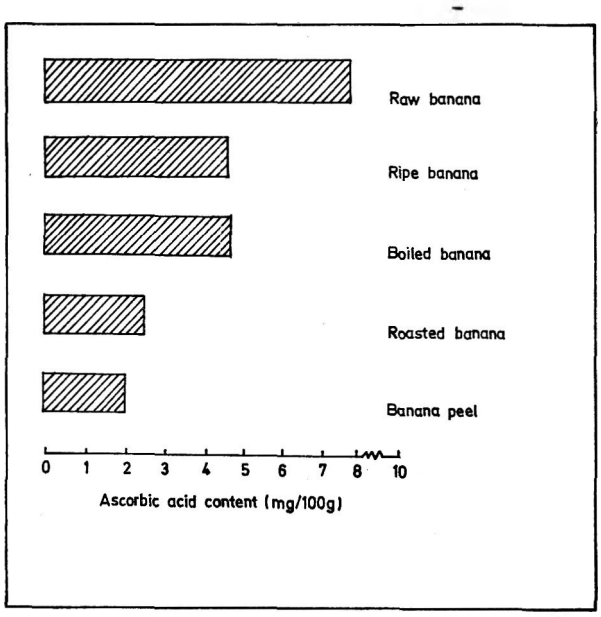

Fig 2. Ascorbic acid content of 'malindi' banana.

Roasting the banana to a temperature of 66°C, lowered the ascorbic acid content by 65 per cent, whereas boiling for 30 min lowered it by 35 per cent. As shown in Fig 2. roasting of 'malindi' banana destroyed more vitamin than boiling. The ascorbic acid content of the boiled banana was about the same as that of the fully ripe banana and that of the roasted banana was lowered to the quantity present in the banana peel.

The vitamin C requirement for an adult in Tanzania is estimated to be 65 mg per day[9]. Where banana is a common staple, it is among the major contributors of the vitamin. Consumption of about 3 kg boiled banana per day would provide more than three times the needed quantity of the vitamin. A nutritional survey in Pangani area south of Mt. Kilimanjaro representing over one million population mainly dependent on banana has not revealed any cases of vitamin C deficiency[10]. Of the the few varieties of Tanzanian bananas analysed, 'sukari', in addition to its modest content of vitamin C, has also a very high caloric value of 147 kcal/100 g while the average caloric value for other bananas was only 88 kcal/100 g[11]. It is expected that in areas where banana is not a staple, very little of vitamin C is provided by the bananas, only the dessert type bananas being popular in these areas. A survey in Mwea-Tebera, the region that mainly depended on maize rather than bananas, revealed that the average daily intake of vitamin C was only 21 mg[8]. Used as staple food, the bananas provide sufficient quantity of ascorbic acid in addition to the required calories. Boiled bananas retain the vitamin more than the roasted bananas. As has been suggested, the loss of ascorbic acid can be kept to a minimum by cooking for a short period of time with minimum amount of water.[12]

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USE OF ROLLER DRYER FOR MANUFACTURE OF KHOA

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Roller dryer was used for the manufacture of khoa to develop a process for large scale production. The concentrated milk of about 50-55 per cent total solids prepared in a jacketted steam pan was used in the laboratory scale roller dryer. Steam pressure, rpm of rollers, etc., were adjusted to get khoa type consistency. Organoleptic evaluation of the product showed that colour, body and texture were comparable to khoa made by the conventional method. The flavour and appearance score of the khoa were less than the control product; however the product was acceptable.

The present technology for khoa making is developed to meet the small scale requirement of khoa traders. However in recent years, organized dairy plants have shown their interest to manufacture khoa on a large scale. Rajorhia[1] attempted a semi-commercial process for preparation of khoa in a jacketted steam heated stainless steel kettle. The milk was stirred with the help of a built-in-stirrer. However the stirrer did not function well during the later part of khoa manufacture. Therefore, to overcome the difficulties of the batch method Banerjee and co-workers[2] developed an equipment for continuous process of khoa making. Subsequently, the method of khoa production with this equipment was standardized by Singh and De[3]. The main defect of the finished product was a soggy body. Rajorhia and Srinivasan[4] proposed many alterations in the design of the plant to improve the quality of the product.

The product has a big potential if a suitable method for its manufacture is developed employing existing dairy equipments. The present work was undertaken to study the feasibility of khoa preparation using roller dryer and to judge the quality of the khoa manufactured by this method for its various attributes.

Standardized buffalo milk containing 5.0 per cent fat was used for manufacture of khoa in all the trials, each of 10.0 kg milk. Two types of khoa were prepared by conventional method using unhomogenized (CKUH) and homogenized milk (CKH). Homogenization of the milk was carried out in a single stage homogenizer (Rannie, England) at 140 kg/cm^2 pressure and 45°C For preparation of khoa on roller dryer, the milk was concentrated to 50-55 per cent total solids in a double jacketted stainless steel steam kettle with continuous stirring and was partially dehydrated on a laboratory scale roller dryer having drum diameter and length of 16 and 15 cm respectively and operating at 3.0 rpm and between 1.5 and 1.8 kg/cm^2 steam pressure. Partially dried material having khoa like consistency was obtained. The material was worked to get pat form of the product. Two types of khoa were prepared on roller dryer using unhomogenized (RKUH) and homogenized (RKH) milk. Homogenization of the milk was carried out using similar homogenizer under the same conditions as used in the conventional method.

Khoa prepared by different methods was given to a judging panel consisting of 8-10 semi-trained judges selected from the staff members of the Faculty of Dairy Science, Anand. Each attribute carried a maximum score of 10. The data for various attributes of the products are given in Table 1.

Colour of khoa samples was not significantly influenced by the different methods of khoa making. The colour score of RKUH (7.33) was slightly less than CKUH (8.09). Some judges commented that there was excessive free fat in RKUH. However homogenization of the milk improved the colour; it gave a whiter appearance and reduced the presence of visually observed free fat on khoa pats. Further, it was noticed that RKUH and RKH were rated as good as those of respective control samples prepared by conventional method.

Although homogenization of milk had improved the scores for body and texture, they were not significantly different at 5 per cent level. It is visualized that proper blending of scraped material from the rollers was an essential factor to get uniform body and texture of the product.

TABLE 1. ORGANOLEPTIC SCORE OF KHOA PREPARED BY DIFFERENT METHODS*

Type of khoa	Colour	Appearance	Body	Texture	Flavour and taste	Total score
			Conventional method			
Unhomogenized milk	8.09	7.34	8.05	8.18	8.79	40.45
Homogenized milk	8.52	6.08	8.25	8.58	8.76	40.19
			Roller dried			
Unhomogenized milk	7.33	7.07	6.65	6.80	7.52	35.37
Homogenized milk	8.59	7.28	7.23	7.42	7.44	37.96
lsd at 5 %	NS	1.17	NS	NS	0.52	—

*Each value is an average of 3 trials of 8-10 judges.
NS: Not significant.

Flavour and taste scores of khoa prepared by roller dryer were significantly lower than the conventionally prepared khoa samples. Homogenization did not improve the flavour and taste of khoa. However the product was acceptable. It is concluded that khoa having acceptable quality can be prepared on roller dryer.

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MIGRATION OF LEAD INTO *DAHI* PREPARED IN EARTHEN CONTAINERS

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***Dahi*, the Indian milk fermented product corresponds to yoghurt and cultured butter milk of Europe. Migration of lead into *dahi* stored in earthen containers as observed by atomic absorption spectrophotometer was found to be well below the permissible limits.**

Dahi is an important fermented milk product of the Indian sub-continent. It has a mildly sour and pleasant flavour formed by the conversion of lactose into lactic acid and volatile flavour forming substances due to bacterial action. When made in earthen containers it has good quality attributes, like body, texture and flavour. Yet the possibility of lead migrating into *dahi* constitutes a health hazard.

Monier Williams[1] found that foods cooked in lead glazed earthenware can take up 3 to 4 ppm of lead and he mentioned that nearly all the pottery used for cooking is made from non-lead glazes in U.S.A. Djuric[2] reported the solubilization of lead from lead glazed pottery in yoghurt. Lead is one of the most toxic elements and effects are cumulative in repeated doses[3]. In human beings it causes serious symptoms of poisoning like behavioural disorders, muscular paralysis, convulsions and permanent brain damage. FAO/WHO Joint Expert Committee reported the provisional tolerable weekly intake of 3.0 mg per person or 0.005 mg per kg body weight. It has been found that 2.0 mg of lead absorbed daily brings about functional disorders in kidneys and arteries[4]. In view of the above, the presence of trace minerals in *dahi* prepared and kept in earthen pots was studied.

Freshly pooled cows milk was collected from the National Dairy Research Institute Dairy and standardised to 4.5 per cent fat and 9.5 per cent solids-not-fat. Mixed starter culture containing *Streptococcus lactis* (S_1R). *Streptococcus cremoris* (SC_1), *Streptococcus diacetylactis* (DRC_1) and *Streptococcus thermophilus* S.W. (Wisconsin) was used. Earthen containers of 250 ml capacity baked to grey and red colour were collected from pottery works in rural and urban areas. Samples of raw clay and baked clay pots made from different sources of clay, were also collected for analysis. Grey colour pots were prepared in a closed kiln while the red coloured ones were prepared by ventilating the red

hot pots by opening the kiln. Glass bottles and earthen containers used in the experiments were cleaned thoroghly in distilled water to eliminate surface trace elements contamination. *Dahi* was prepared by boiling the milk, cooling to 40°C and inoculating with mixed lactic culture at 2 per cent level. The inoculated milk was dispensed into different containers and incubated at 30°C until setting (8 hr).

One gram of well mixed sample of *dahi* which was stored in earthen containers for 14 hr was weighed into a 100 ml conical flask. It was digested first with concentrated nitric acid at room temperature and then with ternary acid mixture (conc HNO_3, H_2SO_4, and 60% $HClO_4$ 10:1:4) at 180-200°C. Blank digestion was run with reagents added in the same amount and order as for the sample. Lead and other trace minerals in the digest were determined in a Perkin-Elmer 403 Model atomic absorption spectrophotometer. The instrument was set at 283.0 mμ and 1 to 2 ppm of lead nitrate solution was used as a standard.

The values of the concentration of heavy metals and other trace minerals are presented in Table 1. It has been found that lead content ranged between 30 and 100 ppm According to Swine[5], soil samples from industrial area contained 5.25 mg of lead/kg of soil while Fairey and Grey[6] reported the presence of 2 mg/kg. Leeper[7] reported the ranges of trace elements in soils: Cobalt 1 to 40 ppm, copper 2 to 4 ppm, lead 2 to 100 ppm, nickel 10 to 1000 ppm, zinc 10 to 300 ppm. It is observed that lead and other metal contents have increased on baking and the metals were present in a higher concentration in urban samples than the rural clay samples and may be attributed to pollution with industrial water.

TABLE 1. HEAVY METALS (PARTS PER MILLION) IN RAW AND, PROCESSED CLAY SAMPLES*

Sample	Type	Concentration of cations				
		Pb	Cu	Zn	Ni	Co
A	Raw	60	97	127	160	80
(Rural)	Grey baked	30	82	71	50	30
	Red baked	50	123	132	20	90
B	Raw	60	96	136	250	90
(Rural)	Grey baked	60	108	137	330	95
	Red baked	55	97	144	260	100
C	Raw	60	104	240	260	80
(Pro-	Grey baked	100	128	650	200	90
cessed)	Red baked	90	128	395	350	115
D	Raw	60	113	144	225	100
(Pro-	Grey baked	70	125	153	240	85
cessed)	Red baked	60	135	154	270	110

*Average of three samples.

TABLE 2. LEACHING OF LEAD INTO *DAHI* AND BUFFER STORED IN GLASS BOTTLES AND EARTHEN CONTAINERS

Product stored	Storage containers	pH	Lead (ppm)
Milk*	—	6.80	0.770
Dahi	Glass bottles (control)	4.80	0.770
	Earthenware	4.68	1.743
Acetate buffer	Glass bottles	4.68	0‡
(pH 4.68)]	Earthenware	4.70	0‡

*Raw milk (control) was used for *dahi* making.
Values are average of three samples.
‡Instead of lead, cations like Ni^{+}, Co^{++}, Cu^{++}, and Zn^{++} were detected.

Dahi stored in earthen containers for 14 hr recorded 1.743 ppm of lead and pH of 4.68, whereas buffer stored in both the containers did not show any migration (Table 2).

Murthy *et al.*[8] reported an average of 0.049 ppm of lead in market milk samples. The extent of lead migrating into *dahi* observed in earthen containers is less than the values of 2 to 4 ppm reported for foods stored in earthen containers and is far below the limit of 7 ppm put forth by Food and Drug Administration of U.S.A.[9]

The authors express their gratitude to Dr. K. K. Iya, Emeritus Professor, UAS for taking keen interest in this project. They thank the Director, National Dairy Research Institute, for providing the facilities and the staff of Geological Survey of India, Bangalore for quantifying the heavy metals.

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THE EFFECT OF AMINO ACID OR PROTEIN SUPPLEMENTATION ON THE NUTRITIONAL QUALITY OF GRAIN AMARANTH

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The supplementary effect of 0.2% L-leucine (reportedly the most limiting amino acid) and 0.2% L-threonine, added to amaranth (*Amaranthus hypochondriacus*) singly was studied in weanling rats. The effect of addition of 2% skimmed milk protein to 6.5% of amaranth protein was also investigated. The results showed that neither 0.2% L-leucine nor 0.2% L-threonine had any significant effect on the net protein ratio (NPR) or relative net protein ratio (RNPR) of grain amaranth. However, supplementation with 2% skimmed milk protein significantly increased the NPR, RNPR of grain amaranth. This latter observation is of practical significance in the development of weaning foods based on amaranth grain.

Grain amaranth (*Amaranthus hypochondriacus*) a hitherto neglected crop of the ancient American Indians, has attracted a great deal of attention in recent years because of the finding that it combines high protein content with high lysine content, an essential amino acid limiting in most cereal grains[1]. The crop is grown in India in the sub-Himalayan ranges and in the Nilgiri hills of South India under the common name of 'ramdana' or 'rajkeera'[2]. The grains are generally popped and made into confection or consumed in the form of pancakes. The protein quality of grain amaranth *per se* was found to be high (75 per cent of that of casein) and it had a good supplementary effect on wheat proteins[3]. The limiting amino acid of amaranth protein, evaluated on the basis of FAO[4] provisional amino acid scoring pattern, is reported[5] to be leucine.

The present study was undertaken to test whether leucine is indeed the limiting amino acid of grain amaranth and also to see if its protein quality could be further augmented by either amino acid or protein supplementation.

Amaranth grain was purchased in bulk quantities from the local market. After cleaning and sieving to remove dust and small grains, it was popped commercially to get well-popped grains. These were powdered fine in a hammer mill for incorporation into experimental diets.

Weanling male albino rats of CFY strain (a fast growing species) were housed in individual, raised, screen-bottomed cages and fed food and water *ad libitum*.

The composition of the diets for various experimental groups is shown in Table 1. The diets were adujsted to a protein level of 8.5 per cent. L-leucine and L-threonine were added singly at 0.2 per cent level to two groups. In another group, amaranth protein 6.5 per cent was supplemented with 2 per cent milk protein. As indices of protein quality, Net Protein Ratio (NPR), and Relative Net Protein Ratio (NRPR), were determined according to the procedure outlined by Pelletand Young[6].

Weight and food intake records were kept for two weeks after the experiment was terminated.

It is apparent from Table 2 that neither L-leucine nor L-threonine when added at 0.2 per cent level in the diet had any supplementary effect on NPR or RNPR of grain amaranth. This shows that neither of these two amino acids is limiting in this grain. Elias *et al.*[7] found no significant response from leucine in their supplementation studies of amaranth. From our earlier studies[3] we had ruled out the supplementary effect of methionine, which, however, Elias *et al.*[7] found limiting in their amaranth species from Central America. Although leucine is reported to be the limiting amino acid of amaranth protein, based on theoretical grounds, the experimental results do not support this contention.

In the present study, skimmed milk protein (2 per cent) significantly enhanced the protein quality of grain amaranth, the RNPR increased from 66 to 78 per cent (Table 2). This may not be surprising since milk proteins are known to improve the protein quality of most plant proteins[8]. However, these results suggest that some essential amino acid other than L-leucine, L-threonine, L-methionine may be limiting in grain amaranth. Further studies are required to determine the amino acid(s) which is (are) limiting in this grain protein.

The results of this study also confirm our earlier observation[11] that there is no change in protein quality of grain amaranth after popping.

The demonstration that a small supplement of skimmed milk protein further improved the protein nutritional value of grain amaranth is of practical significance in the development of weaning food based on this grain which, even otherwise, has a high nutritive value.

TABLE 1. PERCENTAGE COMPOSITION OF EXPERIMENTAL AND CONTROL DIETS

Ingredient	Casein	Protein free	Amaranth				
			Raw	Popped	Popped + L-threonine	Popped + L-leucine	Popped + skimmed milk powder
Casein	10.3	—	—	—	—	—	—
Amaranth (unpopped)	—	—	59.6	—	—	—	—
Amaranth (popped)	—	—	—	59.6	59.6	59.6	45.1
Skimmed milk powder	—	—	—	—	—	—	5.9
DL-methionine	0.3	—	—	—	—	—	—
L-threonine	—	—	—	—	0.2	—	—
L-leucine	—	—	—	—	—	0.2	—
Groundnut oil[+]	10.0	10.0	6.2	6.2	6.2	6.2	7.0
Salt mixture[++]	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin mixture[10]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Choline chloride*	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Cellulose	1.5	1.5	—	—	—	—	—
Starch	72.7	83.3	29.0	29.0	28.8	28.8	36.8

[+]Groundnut oil fortified with vitamins A and D
[++]Salt mixture U.S.P. XVII (9)
*Choline chloride—50% aqueous solution

TABLE 2. EFFECT OF AMINO ACID AND PROTEIN SUPPLEMENTATION ON NET PROTEIN RATIO (NPR) AND RELATIVE NPR OF GRAIN AMARANTH

Group[+]	Protein level (g%)	Initial wt. (g)	Wt. gain (g/2 weeks)	Food intake (g/2 weeks)	NPR	RNPR
Casein+0.3% DL-methionine	8.4	60	86.9 ± 1.78^{a}	179.1 ± 2.86^{a}	6.10 ± 0.132^{a}	100
Amaranth (raw)	8.6	60	37.6 ± 1.15^{b}	124.3 ± 2.36^{b}	4.225 ± 0.062^{b}	69
Amaranth (popped)	9.0	60	43.7 ± 3.10^{bc}	143.2 ± 5.52^{ce}	4.004 ± 0.116^{b}	66
Amaranth (popped)+L-threonine	9.2	60	48.3 ± 2.65^{cd}	150.1 ± 5.49^{cd}	4.093 ± 0.067^{b}	68
Amaranth (popped)+L-leucine	9.2	60	39.7 ± 3.63^{b}	130.5 ± 7.03^{be}	4.016 ± 0.102^{b}	66
Amaranth (popped)+skimmed milk powder	8.8	60	55.5 ± 1.99^{d}	151.0 ± 3.14^{cd}	4.768 ± 0.071^{c}	78

[+]6 rats per group
Values are mean $\pm$ SE
Statistical analysis (analysis of variance)
Groups not having the same superscript were significantly different from each other $P < 0.05$.

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BOOK REVIEWS

Interactions of Food Components: Edited by G. G. Birch and M. G. Lindley, Elsevier Applied Science Publishers, London and New York, 1986; pp. 343; Price, £ 42.00.

The above book is a collection of the papers presented at an Industry-University cooperation symposium organised by the National College of Food Technology, Department of Food Technology, University of Reading, 2-4 April, 1985. It contains sixteen review papers on different aspects of interactions involving sensory and nutritional components, water and food components, macromolecules and metal interactions, nitrites and food stuffs, phenol-protein interactions, and protein-lipid interactions. These papers are edited, indexed and published in the form of a book. The authors of some of the papers presented are well known in their field of specialization. It contains 83 tables and 103 illustrations.

The paper on interaction of water with food components, highlights the role of water activity in food systems and its effect on spoilage. Measurement of these forms of water by a non-thermodynamic technique like NMR is discussed. It would have been appropriate if the latest status of water structure was also discussed in this chapter with a thermodynamic background. There are several papers on the interactions of macromolecules like proteins, carbohydrates, etc. with mineral consituents especially with iron. These highlight isolation of the complex form, measurement of such interactions by physicochemical methods and binding studies. Some of these papers could have been grouped together and a common introduction would have highlighted these interactions in a much better way. The third group of papers comprises interactions of small molecules like sterols, sulphur dioxide, nitrites and phenols with food systems as a whole especially with proteins and lipids. These papers discuss the effect of solution conditions such as concentration of small molecules, pH, temperature, role of moisture and bacterial contamination as an interacting factor. An understanding of these would help to prevent deterioration of specific foods and extending their shelf life. The paper on physicochemical interactions involved in aroma transport process from solution by Darling *et al.*, has been firmly based on mathematical approach and is a unique paper. The authors have correlated surface regeneration behaviour with partitioning and diffusion processes through mathematical relationships in order to understand and predict the transport phenomenon.

It is felt that such a collection of heterogenous papers on a homogenous topic like 'interactions' needed a good introduction which would have introduced any reader to this rather less understood subject in food systems. The book is welcome since not many books are available on this topic.

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Solar Energy for Refrigeration and Air Conditioning: International Institute of Refrigeration, 177, Boulevard Malesherbes F-75017, Paris, France, 1982–1983; pp. 356; Price: 130 FF.

The book contains the proceedings of the commissions E1 (Air conditioning) and E2 (Heat pumps and energy recovery) of the International Institute of Refrigeration held in Jerusalem, Israel. In view of the global energy crisis, the meetings were specially convened to study refrigeration processes employing solar energy. The participants were from Fed. Republic of Germany, Argentina, Australia, Belgium, Brazil, Denmark, USA, France, India, Israel, Italy, Norway, Netherlands, Singapore, Sweden and Venuzuela. The proceedings are presented in six sections.

Section one deals with thermodynamic properties of binary and ternary mixtures (LiBr and or Zn Br_2 solutions in methanol, water) whereas section two deals with performance of solar absorption systems. Other solar refrigeration systems, presented in third section, include vapor jet vapour compression hybrid systems for congeneration of cold and heat using solar energy and performance of four refrigerators with compressors and photovoltaic cell. Section four is devoted to solar heat pumps. The heat delivered by compression and absorption heat pumps originating from the surroundings is also dealt in this section. Section five and six deal with solar collectors and available energy and architectural problems.

The meetings, discussions and conclusions are very constructive. Also contacts and linkages established by the participants with Israelian researchers and engineers whose contributions are well known in the field of solar energy, will be of immense help in solving refrigeration problems of the third world. The book is

recommend for the benefit of those, who are engaged in work pertaining to alterante source of energy and R & D personnel engaged in post harvest preservation of foods by refrigeration.

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

Management of Information Centres in China: by K. P. Broadbent (Ed)., International Development Research Centre, Box 8500, Ottawa, Canada, 1984; pp. 470;

This compilation is the result of a course held in Kunning, Yunnan Province, Peoples Republic of China, 6-18 December 1982, jointly sponsored by the Institute for Scientific and Technical Information in China (ISTIC) and the International Development Research Centre (IDRC) of Canada. The compilation contains lectures given by information specialists from United States, Canada and U.K. Participants from different institutes in 24 provinces and municipalities and specialised information institutes in China participated to get acquainted with the latest developments and techniques in information science.

The contents of the publication under review contain the lectures given by specialists covering various aspects of the management of Information Centres like Function planning and management; budgetary and physical control; facility planning and administration; improving bibliographic services; systems analysis and design; information technology; sources of information and personnel development and performance evaluation. International information systems like MINISIS, AGRIS and CODOC have been been reviewed. Editor himself has taken major share in these lectures (as knowledge of Chinese is needed) followed by M. Beekman, HWA-Wei Lee, J-B. Wills, Olga Landvay and T. C. Ting, The lecture even touches minute aspects covering both theoretical and practical aspects. The lectures included are quite informative and useful for those who want to get acquainted with the latest developments in planning, organisation and management of information systems and services.

K. A. RANGANATH
C.F.T.R.I., MYSORE.

AFST(I) News

Lucknow Chapter

The 6th General Body meeting of the above Chapter was held on 22nd August 1986. The following office bearers were elected unanimously for the year 1986–87.

President	:	Mr. N S. Bisht
First Vice President	:	Mr. O. S. Khanna
Second Vice President	:	Mr. K. G. Shukla
Hony. Secretary	:	Mr. Rajveer Singh
Joint Secretary	:	Mr. Raj Bali Singh
Hony. Treasurer	:	Mr. S. R. Tripathi
Executive Members	:	1. Mr. J. N. Chaurasia
		2. Dr. I. C. Shukla
		3. Dr. J. D. Seth.

ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS (INDIA)

CFTRI Campus, Mysore–570 013

VII INDIAN CONVENTION OF FOOD SCIENTISTS AND TECHNOLOGISTS

Theme: Present Status and Future Perspectives in Technology of Foodgrains

27 FEBRUARY TO 1 MARCH 1987

at

Central Food Technological Research Institute, Mysore-13

The Association has decided to convene the VII ICFoST on 27 February to 1 March 1987 at the Central Food Technological Research Institute, Mysore. The focal theme of the convention will be 'Present Status and Future Perspectives in Technology of Foodgrains'. A full-length symposium on the above subject will be the main activity of the convention.

Our country is at the cross-roads of cereal grains. India produces a diversity of foodgrains in different agro-climatic regions. Rice constitutes roughly 40% of our foodgrain production, some 30% is contributed by wheat and 20-25% by a variety of coarse grains like maize, jowar, bajra and ragi. In addition the Indian sub-continent is the only place where pulses are regularly consumed as a side dish. We thus have a rich mosaic of grain use. Grain is consumed not only as a staple, but also as a variety of snacks and special dishes.

There has been a substantial spurt in the production of food grains in India during the last two decades. Spectacular increase has occurred in the production of wheat, followed by that of rice, jowar and maize, although unfortunately the production of pulses has remained stagnant.

The spurt in production has made great demands on post-harvest technologies including handling drying, storage, milling and processing. Rising income has also caused greater demand on safe, hygienic foods of diverse taste and also ready-to-eat foods. There has been much accumulation of experience in these areas and also a rapid growth in the knowledge of science and technology of processing of food-grains. Therefore it is an opportune time to take stock of the current status and of the future perspectives of the technology of foodgrains, so that we can derive the greatest benefit from the increasing trend of nutritious and palatable food to our consumers.

The subject will be discussed in different sessions for two to three days. Reputed experts will be invited to present theme papers on various topics of interest. The subject would be of interest not only to

* food scientists and technologists but also to those engaged in
* Government administration and policy
* drying, milling and primary processing of the grains
* making baked, confectioned, snacks and other value-added products
* the public-sector agencies and trade who perform the gigantic task of handling, storage and distribution.

Intereaction among experts from these different organizations is likely to be extremely fruitful in charting our future policy. Besides the central theme, lectures of general interest would be arranged.

Poster sessions would be held covering all the aspects of Food Science and Technology.

The Association is the apex body of those engaged in industry, policies as well as R & D in the area of food science and technology. It has successfully organized numerous symposia and conferences in the past on subjects of national importance which have yielded much valuable results. The present one, the seventh in the series of Indian Conventions, is being organized in the same tradition.

The Convention will be held in the enchanting garden city of Mysore. To both those who have experienced its charms and those who have not, its attraction as a prime tourist spot remains ever green. The weather during the Convention time would be pleasant.

AFST(I) cordially invites you to take part in the rich fare that we hope to arrange.

Dr. T. R. SHARMA
President

NAGIN CHAND
Hony-Exec. Secretary

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) $\times$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.

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Research Papers

INTERACTIONS OF PONCEAU 4R WITH COPPER AND EFFECT OF FEEDING PONCEAU 4R ON IRON METABOLISM *by D. Gautam, R. K. Sinha and D. B. Milne*

STUDIES ON THE EFFECT OF PONCEAU 4R ON ^{59}Fe RETENTION IN RATS AND ON PONCEAU 4R—IRON INTERACTION *by R. K. Sinha, D. Gautam, T. J. Zimmerman, T. G. McLeod and F. H. Nielsen*

SUPPLEMENTARY VALUE OF BEET (*BETA VULGARIS*), KNOL-KHOL (*BRASSICA OLERACEA*) AND TURNIP (*BRASSICA RAPA*) GREENS ON THE PROTEIN QUALITY OF WHEAT *CHAPATI by Balwinder Sawhney and B. L. Kawatra*

DEVELOPMENT OF WEANING FOODS BASED ON SOME TRADITIONAL TECHNOLOGIES *by N. G. Molleshi, S. N. Raghavendra Rao, S. Sreedharamurty and H.S.R. Desikachar*

PROTEIN DRINK FROM FISH PROTEIN ISOLATE *by Suvendu Bhattacharya and A. N. Bose*

NUTRITIONAL EVALUATION OF FRACTIONATED RANDOMISED PALM OIL AND VANASPATI *by S. Majumdar A. C. Bhattacharyya and D. K. Bhattacharyya*

Research Notes

A NOTE ON SUPERCRITICAL CARBON DIOXIDE DECAFFEINATION OF COFFEE *by K. Udayasankar, B. Manohar and A. Chokkalingam*

PUFFING QUALITY CHARACTERISTICS OF SOME RAGI (*ELEUSINE CORACANA*) CULTIVARS *by S. S. Shukla, O. P. Gupta, Y. K. Sharma and N. S. Sawarkar*

COMPARISON OF RHEOLOGICAL CHARACTERISTICS OF COW AND BUFFALO CREAM BUTTER *by Satish Kulkarni and M. K. Rama Murthy*

A STUDY OF THE CONCENTRATION OF COPPER, ZINC AND MERCURY IN GOAT'S KIDNEY AND LIVER SAMPLES *by K. Ayyadurai and V. Krishnasamy*

CHANGES IN MUSCLE LIPID COMPOSITION OF WHITE POMFRET (*STROMATEOUS CINEREUS*) DURING ICE-TEMPERATURE STORAGE *by B. Y. Krishnoji Rao and C. Bandyopadhyay*

SPECTROPHOTOMETRIC DETERMINATION OF FENITROTHION AND METHYL PARATHION IN FORMULATIONS AND ITS RESIDUES ON VEGETABLES AND FRUITS *by C.S.P. Sastry and D. Vijaya*

STUDIES ON THE CHEMICAL AND NUTRITIONAL CHANGES OF BAMBOO SHOOTS DURING FERMENTATION *by S. Pravabati Devi and H. Tombi Singh*

EFFECT OF HEAT TREATMENT ON TRYPSIN INHIBITOR AND HAEMAGGLUTININ ACTIVITY IN GUAR MEAL (*CYAMPOSIS TETRAGONOLOBA*) *by S. Ramamani*

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