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Storage Changes in Cookability of Pulses from Three Regions of Andhra Pradesh

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Storage changes in cooking time and water uptake of four pulses namely, green gram, red gram, black gram and Bengal gram stored for one year by rural families of three regions of Andhra Pradesh (Telangana, Coastal and Rayalaseema) have been assessed. Periodical evaluation revealed that green gram took least time and Bengal gram the highest time for cooking. But the maximum increase in time taken for cooking stored pulses was observed in green gram (52%) followed by black gram (35%) Bengal gram (31%) and red gram (29%). Though a progressive decrease in water uptake was observed in stored pulses, no relationship was observed between percentage decrease in water uptake and percentage increase in cooking time. There was a decrease in weight and volume of raw and cooked pulses with the increase in storage period.

Though a major proportion of the pulses consumed in India is in the form of dhal (dehusked split cotyledons) it is also sometimes consumed as whole gram. From the consumer point of view, it is very important that the maximum softening as well as maximum volume of cooked product must be achieved per unit weight or volume of grain.

The time taken for cooking pulses should be minimum. Not only does the time taken for cooking different pulses vary, but varietal difference in cooking quality is also known to exist even in the same pulse[1-3]. Apart from varietal differences, storage condition is also known to affect the cooking quality of pulses[4]. This is a textural problem attributed to the failure of beans to soften sufficiently during the normal cooking process. It develops in beans stored with high moisture content and high storage temperature, the two conditions acting synergistically[6,7].

Hence the present study was planned to assess the effect of storage on time taken for cooking and water uptake of stored pulses collected from three regions of Andhra Pradesh.

Materials and Methods

Red gram (*Cajanus cajan*), green gram (*Phaseolus aureus*), Bengal gram (*Cicer arietinum*) and black gram (*Phaseolus mungo*) were collected from three villages in three regions of Andhra Pradesh, namely, Telangana, Coastal and Rayalaseema. For each pulse, six households were selected at random in each of the three villages.

Storage conditions in all the households from three regions) were similar i.e., all the pulses were stored in gunny bags. Only local variety of each pulse which is grown in the region was stored by the farmer as whole grain. Two hundred grams of the pulse were collected from the farmers soon after harvest and after 4, 8 and 12 months of storage for analysis.

Cookability of pulses: Cooking time, weight and volume before and after cooking and water uptake were determined as described by Narasimha and Desikachar[5].

Statistical analysis: Analysis of variance was used to find out the effects of storage in different regions and for different periods on the cooking quality of the stored pulses.

Results and Discussion

Effect of storage on cooking time: The cooking time differed significantly ($P \leq 0.05$) among the four pulses and the time required for cooking green gram was the least and for Bengal gram the highest. (Table 1). The time taken for cooking of all the pulses increased significantly ($P \leq 0.05$) with the period of storage. At the

TABLE 1. COOKING TIME OF FRESH AND STORED PULSES

Region	Cooking time (min) after storage at indicated periods (months)			
	0	4	8	12
	Green gram			
Telangana	30±3.4	37±4.1	42±3.6	47±2.2
Coastal	27±2.5	33±1.6	39±3.5	45±3.3
Rayalaseema	35±4.6	42±3.7	44±4.1	49±3.5
	Red gram			
Telangana	65±3.1	73±2.8	75±3.2	86±4.1
Coastal	67±5.9	73±5.9	76±4.0	89±4.1
Rayalaseema	71±6.8	79±3.7	83±4.7	91±3.8
	Bengal gram			
Telangana	75±3.5	80±6.1	83±5.1	95±4.0
Coastal	73±5.7	76±4.3	84±4.8	91±3.2
Rayalaseema	86±6.4	92±7.4	97±6.2	111±7.1
	Black gram			
Telangana	52±2.8	57±3.5	60±3.1	66±2.3
Coastal	52±3.0	58±3.9	62±2.8	74±4.1
Rayalaseema	50±3.5	57±3.8	60±2.7	65±2.9

Mean of 6 samples ± S.D.

end of twelve months storage maximum increase in the time taken for cooking was observed in green gram (52 per cent) followed by black gram (35 per cent) Bengal gram (31 per cent) and red gram (29 per cent). Among the four pulses, green gram which required much lesser initial cooking time than the other pulses showed the maximum increase in cooking time during storage.

Softening of pulses during cooking occurs as a result of gelatinization of starch in the form of deformed starch granule. It has been reported by Sefeh-dedeh *et al*[4] that change in microstructure occurs and breakage of middle lamella leads to softening of cowpeas during cooking. During storage, the moisture, temperature and relative humidity fluctuation might lead to the hardening of this middle lamella and hence more time will be required for its disintegration. Probably such structural change might have contributed to the hard-to-cook defect in the four pulses leading to increase in cooking time.

Effect of storage on water uptake: Table 2 presents the water uptake pattern of fresh and stored pulses. The initial water uptake was different among the pulses. Yet significant differences were not observed in water uptake between Bengal gram and red gram, though there was a significant difference ($P \leq 0.05$) between them in cooking time. Also, green gram which had the highest water uptake (2.03 g/g) required minimum

TABLE 2. WATER UPTAKE OF FRESH AND STORED PULSES ON COOKING

Region	Water uptake (g/g) during indicated period of storage (months)			
	0	4	8	12
		Green gram		
Telangana	1.86±0.17	1.67±0.26 (10.5)	1.54±0.31 (17.7)	1.43±0.21 (23.1)
Coastal	2.11±0.12	2.07±0.13 (2.2)	1.69±0.18 (20.2)	1.37±0.15 (23.1)
Rayalaseema	2.13±0.13	1.86±0.13 (12.6)	1.74±0.13 (18.3)	1.53±0.11 (28.1)
		Red gram		
Telangana	0.83±0.15	0.47±0.05 (42.4)	0.42±0.05 (46.0)	0.37±0.05 (59.5)
Coastal	0.82±0.10	0.40±0.09 (51.7)	0.35±0.09 (57.9)	0.28±0.06 (66.4)
Rayalaseema	0.80±0.10	0.57±0.06 (28.8)	0.39±0.06 (50.0)	0.32±0.02 (59.1)
		Bengal gram		
Telangana	0.79±0.09	0.41±0.05 (47.9)	0.36±0.04 (54.8)	0.27±0.05 (65.7)
Coastal	0.88±0.05	0.47±0.08 (43.9)	0.37±0.04 (58.3)	0.23±0.03 (74.2)
Rayalaseema	0.77±0.10	0.52±0.05 (31.3)	0.37±0.06 (51.4)	0.27±0.04 (64.7)
		Black gram		
Telangana	1.71±0.09	1.53±0.07 (10.3)	1.38±0.08 (18.4)	1.19±0.06 (30.0)
Coastal	1.87±0.10	1.53±0.11 (17.6)	1.31±0.10 (29.6)	1.22±0.08 (34.7)
Rayalaseema	1.62±0.11	1.34±0.07 (17.0)	1.24±0.06 (23.4)	1.08±0.00 (33.1)

Mean of 6 Samples ± S.D.

Numbers in parenthesis indicate per cent decrease on storage.

cooking time whereas Bengal gram which showed the minimum water uptake (8.81 g/g) required the maximum time for cooking. As the period of storage increased, the water absorption capacity decreased significantly in all the four pulses from the three regions. The percentage decrease in water absorption was found to be higher in samples stored in coastal region.

Effect of storage on weight and volume (before and after cooking) of pulses: The weight and volume of fresh and stored pulses are presented in Table 3. Percentage increases in the weight and volume after cooking are presented in Fig 1 and 2.

The initial weight of the four pulses differed significantly ($P \leq 0.05$) depending on the size of the grain. At the end of 12 months storage, the weight of 100 grains before cooking decreased significantly ($P \leq 0.05$) in all the four pulses and samples from Coastal region showed a higher percentage decrease in accordance with the level of insect infestation (as judged by number of insects/100g of sample). Among the four pulses percentage decrease in weight was found to be more in

TABLE 3. WEIGHT AND VOLUME OF FRESH AND STORED PULSES

Region	Wt (g/100 grains)		Vol (ml/100 grains)	
	0	12	0	12
Green gram				
Telangana	3.90±0.2	3.37±0.3	3.59±0.3	3.20±0.2
Coastal	3.66±0.3	2.98±0.4	3.37±0.3	3.07±0.3
Rayalaseema	2.48±0.2	2.00±0.2	2.48±0.2	2.06±0.2
Red gram				
Telangana	7.10±0.4	6.42±0.4	6.97±0.5	6.60±0.4
Coastal	7.60±0.6	6.95±0.4	7.18±0.7	6.76±0.8
Rayalaseema	8.21±0.4	7.67±0.5	7.77±0.4	7.20±0.4
Bengal gram				
Telangana	15.79±1.0	15.64±1.0	15.43±1.1	15.33±0.4
Coastal	15.05±0.9	14.82±0.9	14.17±0.6	13.90±0.6
Rayalaseema	16.06±0.8	15.87±0.9	15.72±0.8	15.48±0.8
Black gram				
Telangana	5.66±0.4	5.46±0.4	5.38±0.6	5.07±0.6
Coastal	5.73±0.3	5.44±0.2	5.58±0.3	5.33±0.4
Rayalaseema	5.63±0.5	5.35±0.5	5.35±0.4	4.95±0.4

Mean of 6 samples ± S.D.

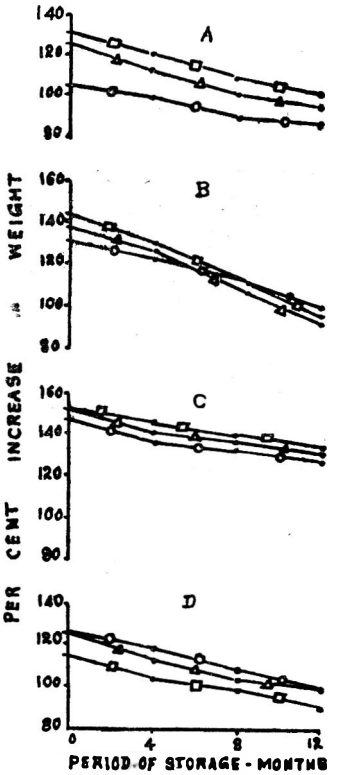

Fig. 1. Percentage increase in weight after cooking of stored pulses. A—Green gram B—Red gram C—Bengal gram D—Black gram. O—O Telangana △—△ Coastal □—□ Rayalaseema.

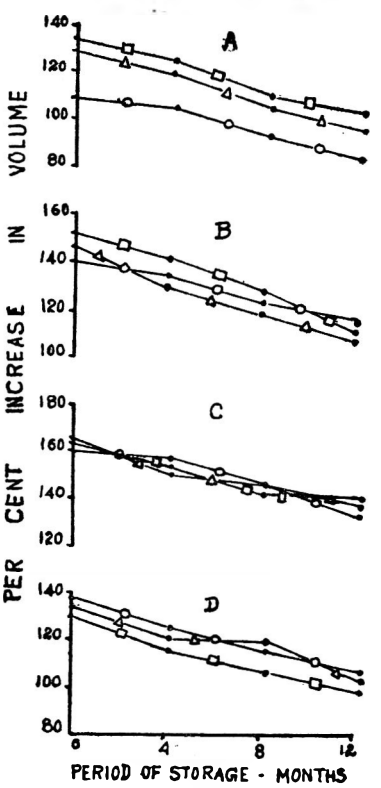

Fig. 2. Percentage increase in volume after cooking of stored pulses. A—Green gram B—Red gram C—Bengal gram D—Black gram. O—O Telangana △—△ Coastal □—□ Rayalaseema.

green gram, followed by red gram, black gram and Bengal gram.

As the weight of the four pulses differed, significant differences ($P \leq 0.05$) were also observed in their initial volumes (before cooking). Though slight decreases in the volume of the pulses from the three regions were observed at the end of 12 months storage, this decrease was not found to be significant, As the insect feeds on the starchy endosperm and leaves the grain intact, the weight of the grain decreases and not the volume. The cooked weight and volume of the four pulses decreased progressively as the period of storage increased. The largest decreases in the cooked weight and volume were observed in red gram, followed by green gram, black gram and Bengal gram (Fig. 1 and 2). The decreased water absorption capacity of the pulse during storage may also affect the cooked weight and volume of the pulse during storage.

The limited data in this study indicate that except for the initial cooking time and water uptake of pulses, there does not seem to exist any relationship between percentage decrease in water uptake and percentage increase in cooking time of stored pulses.

Acknowledgement

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Changes in Physico-chemical Properties of Defatted Soy Flour During Storage

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Defatted soy flour was packed in cloth bag, polycoated jute bag and polyethylene bag and stored for 90 days at 22 and 37°C under 54 and 86% RH. Cloth bag packed samples stored at 37°C under 86% RH were darker, more clumpy with higher microbial load than those packed in polycoated jute bags. The changes in chemical properties were more in the cloth bag packed samples and stored at higher temperature and humidity followed by those packed in polycoated jute bags. The samples packed in polyethylene bags and stored at low temperature and humidities did not show noticeable changes in physico-chemical properties. It was concluded that defatted soy flour packed in polyethylene bags and stored at 22°C under 54% RH could keep well for longer period than those packed in cloth/jute bags and stored at 37°C under 86% RH.

Extraction and refining of soybean oil has become a well established industry and soybean now contributes a significant proportion of edible oil supply in India[1]. The annual production of defatted soy meal, is about 2.5 to 3.0 lakhs tons. A large quantity is exported to Western countries where it is used as cattle feed. It contains about 50-55 per cent good quality protein and can form a part of Indian diets after milling into flour. It can be easily incorporated at 10 per cent level into wheat flour for chapati making without affecting the quality of chapati[2]. However, improper storage causes alterations in physico-chemical, nutritional and functional properties of defatted soy flour and thus renders it unsuitable for consumption. Since information available on the storage of defatted soy flour is scanty, the present investigation was undertaken.

Material and Methods

Defatted soy flour obtained from M/S Prag Ice and Oil Mills Ltd was packed in 50-g quantities per bag in each of cloth, polycoated jute (thickness of polyethylene coating, 28μ) and polyethylene (90μ) bags. The bags were stored at 22 and 37°C under 54 per cent and 86 per cent relative humidities for 90 days. The physico-chemical changes were assessed at intervals of 15 days. Visual observations were made for changes in colour and flow behaviour.

Moisture, protein, fat and total ash contents were determined by standard AOAC methods[3]. N_2-free extract was arrived at by substracting the sum of moisture, protein, fat and ash contents from 100. Water soluble nitrogen and reducing sugars were determined by the AACC methods[4]. Non-protein nitrogen was estimated by the method of Bhatty and Finlayson[5]. Weighed quantity (1g) of sample was dispersed in 25 ml of 10 per cent trichloroacetic acid (TCA) stirred for 1 hr and centrifuged at 5000 r.p.m. for 10 min. The supernatant was transferred to a kjeldahl flask for estimating nitrogen. Available lysine was estimated by Carpenter's method[6]. Urease activity was determined by the modified method of Caskey and Knapp[7] and expressed as the increase in pH over blank. The modified method of Kakede *et al.*[8] was used to determine trypsin inhibitor activity. All the analyses were done in duplicate and the average values have been reported.

Results and Discussion

The defatted soy flour used had the following percentage composition, protein, 55.5; fat, 0.7; ash, 7.1 and carbohydrate, 36.7 on dry weight basis. The initial moisture content was 3.5 per cent. It was observed that the samples of defatted soy flour packed in cloth bags and stored at 22 and 37°C under 86 per cent RH exhibited darker colour and clumpiness along with

microbial contamination after 30 and 15 days respectively, whereas the corresponding periods for the samples packed in polycoated jute bags were 45 and 30 days under similar conditions of storage. Neither clumpiness nor darker colour was observed in soy flour packed in polyethylene bags. At the lower relative humidity (54 per cent) the samples did not exhibit any physical change irrespective of storage temperature and packaging materials. Insect infestation was not observed in any of the sample stored under different conditions.

Moisture content of defatted soy flour during storage increased from an initial value of 3.5 to 21.6 per cent. Samples packed in cloth bag and stored at 22°C under 86 per cent RH showed maximum increase whereas that packed in polyethylene bags and stored at 37°C under 54 per cent RH, exhibited lowest moisture absorption (Fig. 1).

The change in water soluble nitrogen content of defatted soy flour during storage is depicted in Fig. 2. It increased from an initial value of 2.17 to 3.05 per cent during first 15 days of storage but thereafter it declined to the lowest value of 0.19 per cent depending upon packaging materials and storage conditions. The decrease was maximum in samples packed in jute bags and stored at 37°C under 86 per cent RH which might be due to the interaction of protein with sugars as reflected by the development of brown colour. The possible decrease in pH of the flour during storage may also result in low water extractability of proteins. Prolonged storage has also been reported to decrease

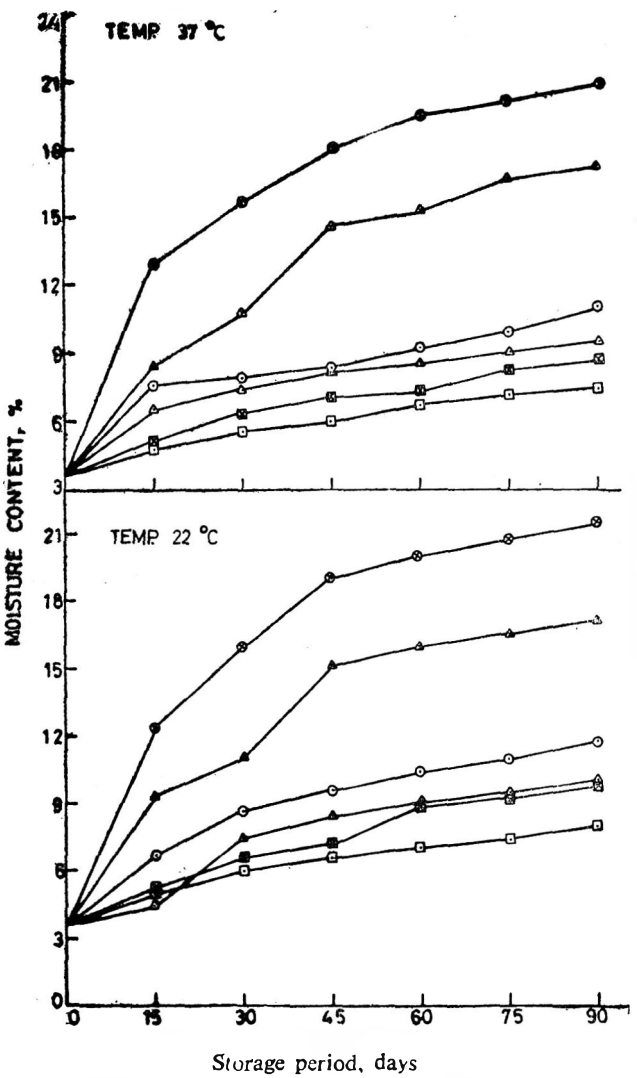

Fig. 1. Changes in moisture content during storage of the defatted soy flour.
R.H. %, Package: ⊙ 54 Cloth Bag, ● 86 Cloth Bag, △ 54 Jute Bag, ▲ 86 Jute Bag, □ 54 Polyethylene Bag, ■ 86 Polyethylene Bag.

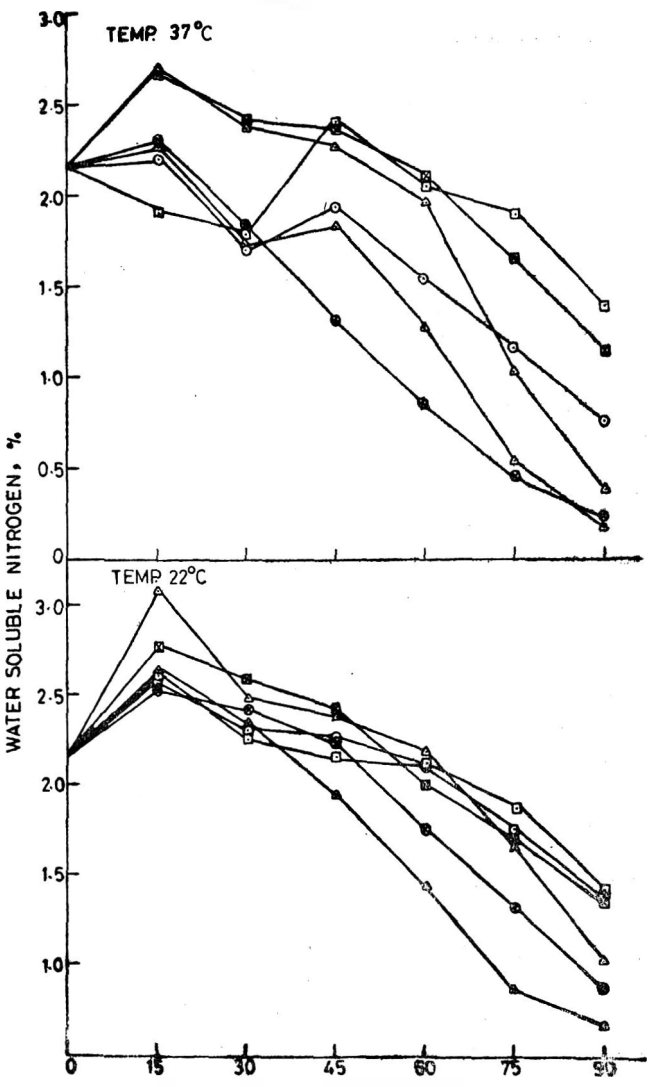

Fig. 2. Changes in water soluble nitrogen during storage of the defatted soy flour.
R. H. %, Package: ⊙ 54 Cloth Bag, ● 86 Cloth Bag, △ 54 Jute Bag, ▲ 86 Jute Bag, □ 54 Polyethylene Bag, ■ 86 Polyethylene Bag.

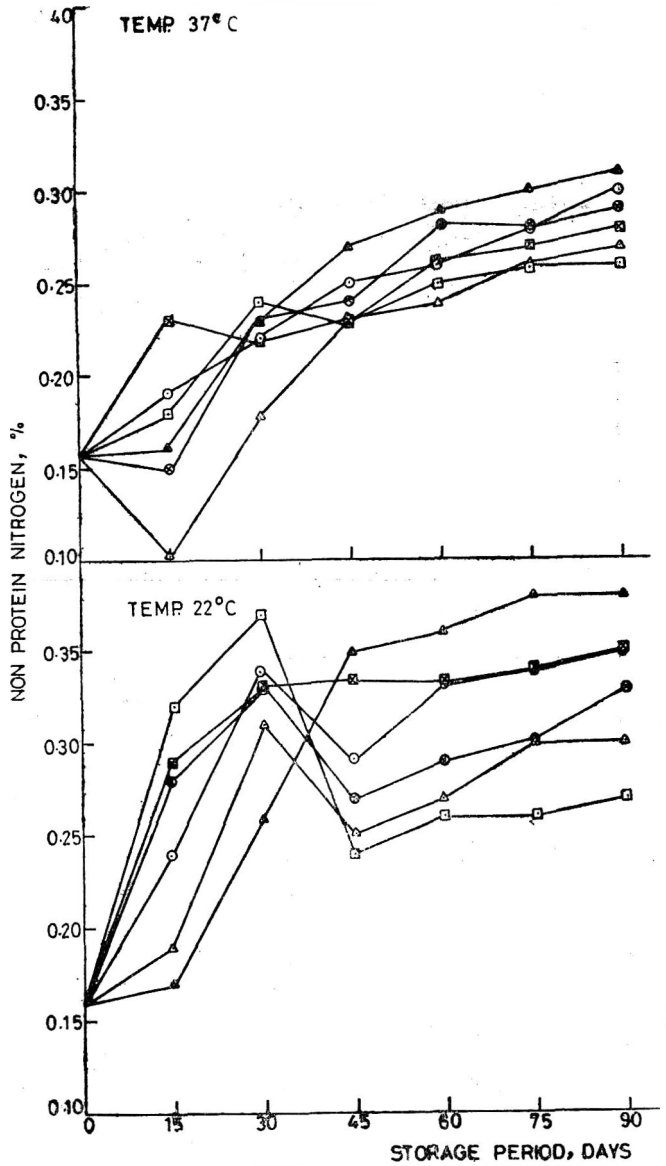

Fig. 3. Changes in non-protein nitrogen during storage of the defatted soy flour.
R. H. %, Package: ⊙ 54 Cloth Bag, ● 86 Cloth Bag, △ 54 Jute Bag, ▲ 86 Jute Bag, □ 54 Polyethylene Bag, ■ 86 Polyethylene Bag.

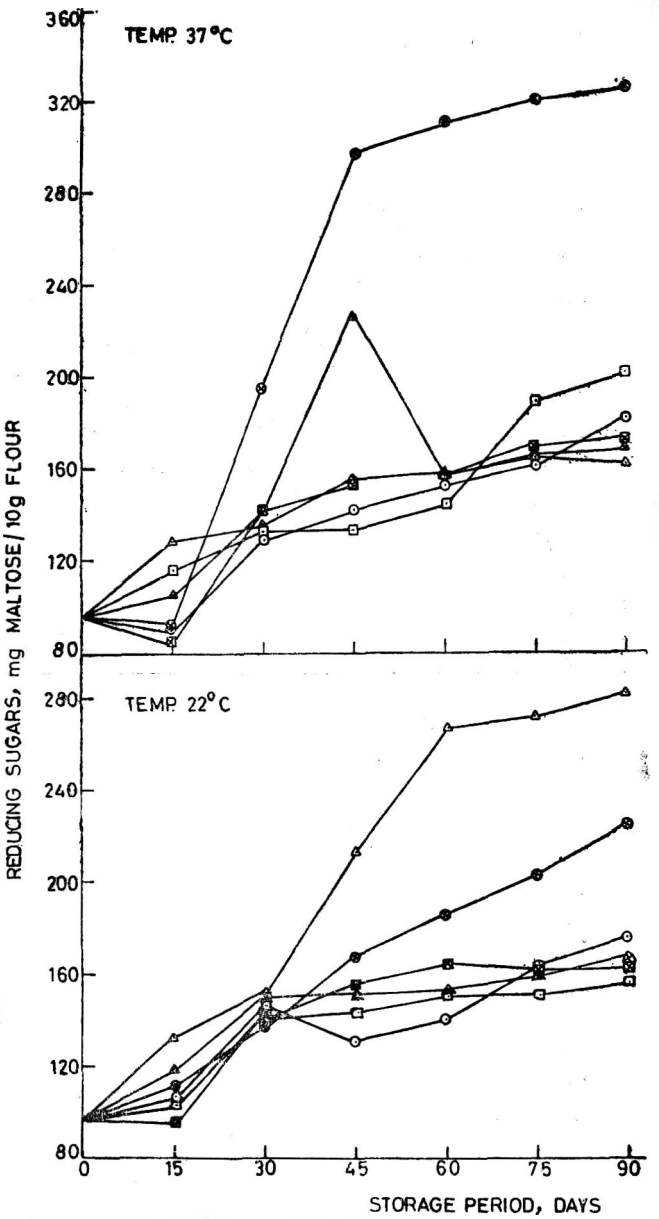

Fig. 4. Changes in reducing sugar during storage of the defatted soy flour.
R. H. %, Package: ⊙ 54 Cloth Bag, ● 86 Cloth Bag, △ 54 Jute Bag, ▲ 86 Jute Bag, □ 54 Polyethylene Bag, ■ 86 Polyethylene Bag.

hydrophillic characteristics of protein molecules in wheat[9].

Non-protein nitrogen content of defatted soy flour increased from 0.15 to 0.38 per cent depending upon the storage conditions and packaging materials. The increase was maximum in the samples stored in jute bags at 22°C under 86 per cent RH and minimum in those stored in polyethylene bags at 37°C under 54 per cent RH (Fig. 3). This indicated that RH is a dominating factor over temperature for this change. The degradation of proteins is probably responsible for the increase in NPN.

The reducing sugar content increased from 96.0 to 325 mg maltose/10 g flour during storage depending upon the storage conditions and packaging materials (Fig. 4). However, this increase was greater in the samples stored in cloth bags at 37°C under 86 per cent RH. The increase in reducing sugars indicated that the complex molecules of carbohydrates get degraded during storage. The increase in urease activity was little without any consistent trend.

The trypsin inhibitor activity (TIA) of defatted soy flour increased during storage whereas available lysine content decreased. The increase in TIA was maximum

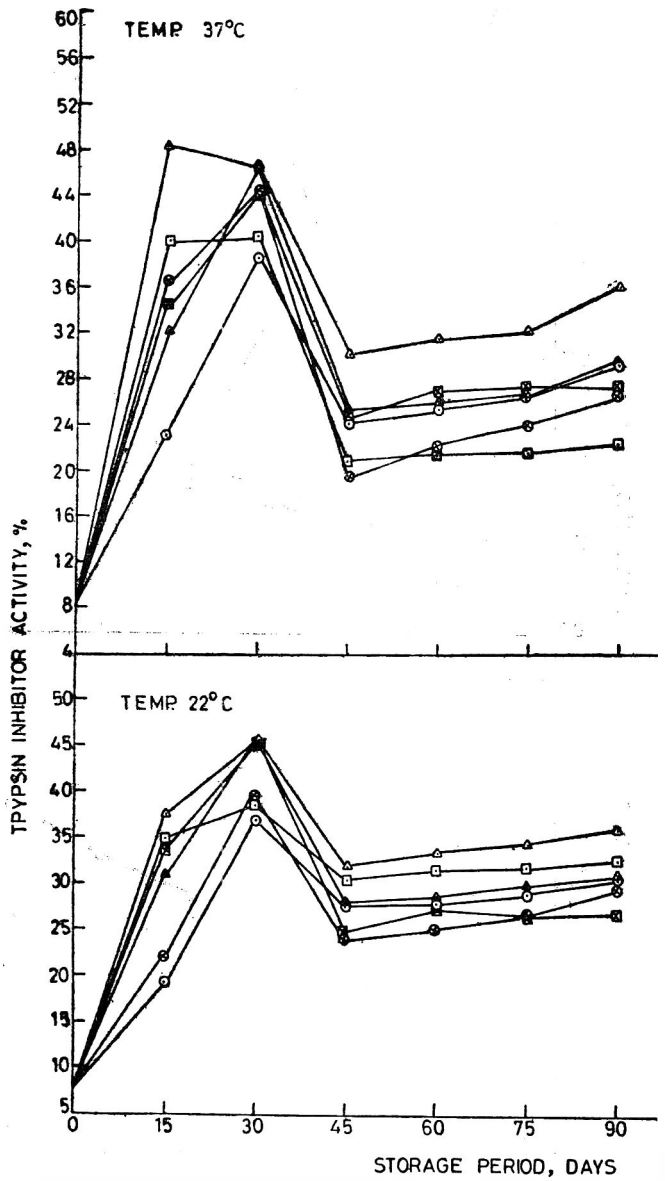

Fig. 5. Changes in trypsin inhibitor activity during storage of the defatted soy flour.

R. H. %, Package: ⊙ 54 Cloth Bag, ● 86 Cloth Bag, △ 54 Jute Bag, ▲ 86 Jute Bag, □ 54 Polyethylene Bag, ■ 86 Polyethylene Bag.

in soy flour stored in jute bags at 37°C under 86 per cent RH and minimum in soy flour stored in polyethylene bags under similar conditions (Fig. 5). On the other hand, the maximum decrease in available lysine i. e. from 5.04 to 4.03 per cent was exhibited by the samples stored in cloth bags under similar conditions of storage (Fig. 6). Yanichek and Iraskova[10] also reported an increase in TIA of soy flour samples stored at room temperature.

From the results, it can be concluded that the samples of defatted soy flour packed in polyethylene bags and

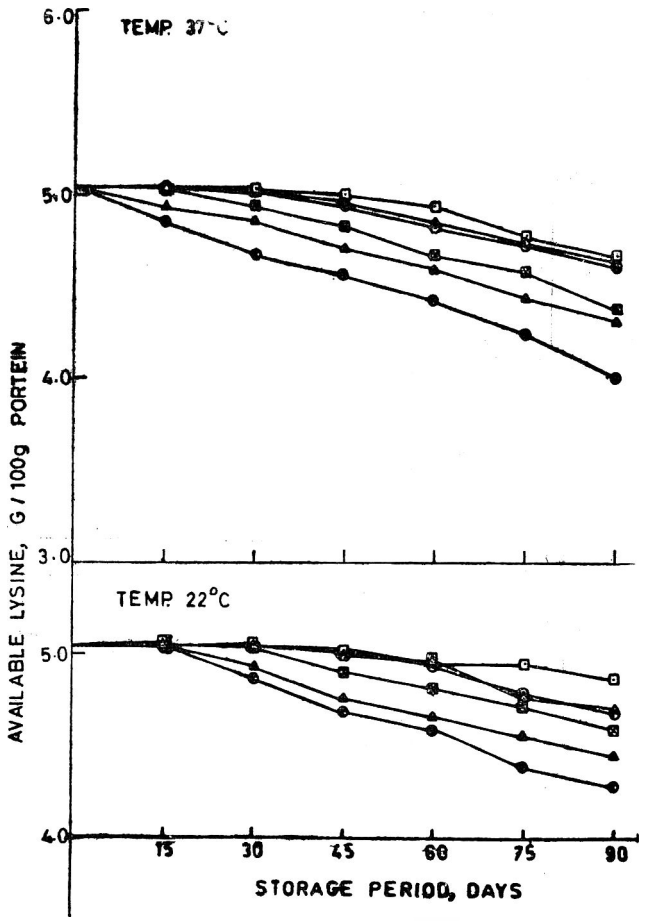

Fig. 6. Changes in available lysine during storage of the defatted soy flour.

R. H. %, Package: ⊙ 54 Cloth Bag, ● 86 Cloth Bag, △ 54 Jute Bag, ▲ 86 Jute Bag, □ 54 Polyethylene Bag, ■ 86 Polyethylene Bag.

stored at 22°C under 54 per cent RH showed minimum changes in their physical and biochemical properties followed by that packed in polycoated jute bags. However, the changes were of greater magnitude when stored at higher temperature and relative humidities.

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A Study of the Microflora at Various Stages of Processing of Fluid Milk in Sri Lanka

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A study was undertaken to ascertain the growth of micro-organisms in milk at various stages of processing. Although raw milk as purchased had a total viable count of 10^6 to 10^7 per ml, processed milk gave a total viable count of less than 5×10^4 per ml. *E. coli* was present in raw milk as well as in some milk samples which were drawn from various points in the processing line. Salmonella was not present at any stage of processing. Bottles washed in washing machines showed lower microbial counts than those from the crates. Washed bottles from the crates were also contaminated with *E. coli*. Poor personal sanitation was observed in employees. Tap water used was free from *E. coli* and showed very low presumptive coliform counts. Swabs from various points in the processing line showed that microbial counts were greatly reduced by sterilization. However, the pipelines and the rubber washers showed substantial microbial load which indicated need for further sterilization.

Milk, in addition to being a nutritious medium, presents a favourable environment for multiplication of microorganisms. These can enter the milk from the interior of the udder, exterior of the cow's body, atmosphere, utensils and from the milker or handler[1].

The important bacteria that occur in milk belong to the families *Lactobacteriaceae*, *Micrococcaceae*, *Enterobacteriaceae*, *Pseudomonadaceae*, and *Bacillaceae*[2]. The availability of food, oxygen, moisture, temperature, pH and inhibitory substances are the most important factors affecting the growth of these micro-organisms[1].

Several diseases are commonly carried through milk and its products. Among them are tuberculosis, typhoid fever, diphtheria, scarlet fever, streptococcal sore throat, undulant fever (brucellosis), infectious hepatitis and various intestinal disturbances found especially among children[3]. The spoilage of milk by microbes results in the alteration of milk components, colour, flavour, gas production and production of toxins[4].

Microbial tests on the dairy plant and its environment would indicate the level of sanitation and potential contamination during processing, while tests on milk contact surfaces would show the efficiency of the cleaning up and sanitizing procedures of the equipment. *Escherichia coli* together with coliform organisms are indicators of sanitation in a dairy plant[5]. Processed (pasteurized) milk should have a total viable count of micro-organisms less than 50,000/ml, and should be free of *E. coli*, salmonella and other pathogenic organisms[6].

The purpose of this investigation was to study the microflora at various stages of processing of milk to understand the effectiveness of each and every step in processing with respect to destruction of micro-organisms, and to adopt corrective measures, if any.

Materials and Methods

While the milk was being processed in a commercial

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dairy plant in Sri Lanka, duplicate samples of milk (10 ml) were drawn aseptically from receiving cans, pasteurizing vats, homogenizer, surface cooler, bowl filler and bottled milk. Duplicate swab samples were also obtained before and after sterilizing from the same area in the pasteurizing vat, homogenizer, condenser between homogenizer and surface cooler, surface cooler, bowl filler, rubber washers, pipeline from homogenizer to cooler, pipe line from cooler to bowl filler, wooden crates, washed and unwashed milk bottles and from hands of three labourers employed at the creamery. In addition, samples of tap water used in the dairy plant were also taken for study.

The total viable count in the samples was estimated using nutrient agar[7]. Qualitative determination of coliform bacteria and *E. coli* was done using brilliant green lactose bile broth (2 per cent), peptone water and Kovac's reagent[8]. Presumptive coliform count and *E. coli* counts were determined using the standard 3 tube M.P.N. method and appropriate media (MacConkey's broth and Brilliant green lactose bile broth) as outlined by Fishbein *et al.*[9] Qualitative determination of salmonella was done using the methods outlined by Poelma and Silliker[10]. Two per cent hot sodium hydroxide (77°C) was used to sterilize the processing line.

To study the effectiveness of pasteurization, the time during which the milk was held at 62.8°C in the pasteurizing vat was checked on several occasions and the time-temperature relationship was established during heating, holding and bottling of the milk. The death rate of bacteria with temperature was also recorded during heating, holding and bottling.

Table 1. TOTAL VIABLE COUNT[a] OF MICROORGANISMS

Source	Total viable count (CFU)[b] 1st Expt.	2nd Expt.	3rd Expt.	4th Expt.
A. Milk (CFU/ml)				
Receiving cans	6.64×10^6	6.27×10^6	7.2×10^6	5.5×10^6
Pasteurizing vats (after pasteurization)	8.0×10^4	4.0×10^4	1.1×10^5	6.0×10^4
Homogenizer	8.0×10^4	3.0×10^4	1.0×10^5	5.0×10^4
Surface cooler	6.0×10^4	2.0×10^4	8.0×10^4	4.0×10^4
Bowl filler	5.0×10^4	2.0×10^4	7.0×10^4	4.0×10^4
Bottled milk	5.0×10^4	3.0×10^4	7.0×10^4	5.0×10^4
B. Wash bottle (CFU/bottle)				
Directly from washing machine	513	520	443	500
From the crates: Before washing with 2% NaOH	826	853	1046	920
After washing with 2% NaOH	213	300	580	280
C. Tap water (CFU/ml)				
Tap water at (28 ± 2°C)	5	36	47	75

[a]Each value was a mean of 10 determinations.

[b]CFU—Colony forming units.

The four experiments were done on different processing dates.

Results and Discussion

Total viable count: The microbial population in the milk varied from stage to stage of processing and significant differences in the total count was observed with the various stages of processing (Table 1).

Initially, the milk in receiving cans contained high microbial population (6.4×10^6 CFU/ml,) owing to the contamination from farms or cattle sheds, during transportation or handling. When milk was pasteurized (62.8°C for 30 min) the total viable count decreased appreciably (7.25×10^4 CFU/ml,) due to the effect of heat treatment, where probably most of the psychrophiles and the mesophiles were destroyed. The total viable count of micro-organisms showed further reduction after surface cooling, which was especially due to the destruction of the thermophilic bacteria. As the cooling process continued, there was a further reduction in the microbial number from the surface cooler to the bowl filler. However, bottled milk showed a slight increase in the total viable count which could be due to contamination from the washed bottles. In this study, the total viable count of the pasteurized bottled milk was mostly below 5×10^4 CFU/ml which satisfies the standards for pasteurized milk (50,000 CFU/ml) specified by the Bureau of Ceylon Standards[6].

The washed bottles taken directly from the washing machine showed different total viable counts, mostly below the maximum specified count of 600 CFU/bottle[6]. But the washed bottles taken from the crates showed higher total colony count than the washed bottles taken directly from the washing machine, and often the values were greater than the specified maximum count. However, when the crates were sterilized with 2 per cent sodium hydroxide, the counts were well below the maximum specified standards.

The values obtained for the microbial count from the tap water samples taken on different occasions (Table 1) were 41 CFU/ml which is below the specified standards of 100 CFU/ml water[6].

Occurrence of coliform and E. coli *in milk*: Milk taken from various parts of the processing line showed different results on different days for the presence of

TABLE 2(a). DETECTION OF COLIFORM BACTERIA AND E. COLI IN MILK FROM DIFFERENT SOURCES

Source of milk	1st experiment		2nd experiment		3rd experiment		4th experiment	
	Coliform	*E. coli*	Coliform	*E. coli*	Coliform	*E. coli*	Colifoem	*E. coli*
Receiving can	+ + +	+ + +	+ + —	+ + —	+ + —	+ + —	+ + —	+ — —
Pasteurizing vats	+ + —	— — —	+ + —	— — —	+ + +	— — —	+ + —	— — —
Homogenizer	+ + —	— — —	+ — —	— — —	+ — —	— — —	+ — —	— — —
Surface cooler	+ — +	— — —	+ + —	+ — —	+ + —	+ + —	+ + —	— — —
Bowl filler	+ + +	— — —	+ + —	+ + —	+ — —	+ — —	+ + +	— — —
Bottled milk	+ + —	— — —	+ + +	+ + —	+ + +	+ + —	+ + +	+ — —

+, detected (3 tube M.P.N.)
—, not detected (3 tube M.P.N.)
The 4 experiments were the mean of 10 separate detections on different occasions.

coliforms and *E. coli* (Table 2a). Milk from the receiving cans always showed the presence of coliforms and *E. coli*, which was again due to the contamination in farms during transportation and handling. In the first experiment, *E. coli* had been effectively destroyed by pasteurization. In the second and the third experiments, milk samples from surface cooler, bowl filler and bottles showed the presence of *E. coli* which was probably due to contamination in the processing line itself. In the fourth experiment, due to contamination from the washed milk bottles, the milk samples from bottles showed *E.* coli

The washed bottles taken directly from the washing machine were free from *E. coli*, while some of the bottles taken from the crates were contaminated with *E. coli* (Tabld 2b). This again indicates the unhygienic condition of the crates and necessitates the washing of crates with a detergent. The Bureau of Ceylon standards[6] specifies that the washed bottles should be free of *E. coli* at all times. When treated with hot 2 per cent sodium hydroxide the washed bottles were observed to be free of *E. coli.*

Swabs from both hands of two labourers working in the creamery showed the presence of *E. coli*, while that from a third labourer was free of *E. coli.* This shows poor personal hygiene of labourers.

To ensure satisfactory personal hygiene of the creamery employees, every person employed should be medically examined to be free from communicable diseases and also for carrier state prior to employment. Strict measures should be taken to disallow any employee suffering from facial injuries, skin infection or wearing a bandage for such injury, from entering the processing area. Employees entering the processing area should wash their hands and feet with disinfectant before commencing work and every time they use the toilets[3].

TABLE 2(b). DETECTION OF COLIFORM BACTERIA AND E. COLI IN WASHED BOTTLES*

Source of washed bottles	1st experiment		2nd experiment	
	Coliform	*E. coli*	Coliform	*E. coli*
Washing machines	+ — —	— — —	+ + +	— — —
	+ + —	— — —	+ — —	— — —
	+ — —	— — —	+ — —	— — —
Before washing crates	+ + +	+ + +	+ + +	+ — —
with 2% NaOH	+ + —	+ — —	+ + —	+ + —
	+ — —	— — —	+ — —	— — —
After washing crates	+ — —	— — —	+ + —	— — —
with 2% NaOH	+ — —	— — —	+ + +	— — —
	+ + —	— — —	+ — —	— — —

*Data for 3 bottles are given.
The 2 experiments were repeated 10 times on different processing dates.
+, detected (3 tube M.P.N.)
—, not detected (3 tube M.P.N.)

Microbial quality of tap water: The water samples tested on different occasions were free from *E. coli* (Table 3). Water from tap number 1 was free from presumptive coliform, while water from tap numbers 2 and 3 showed presumptive coliform counts of 9/100 ml, and 3/100 ml, respectively. Though these counts are higher than the nil counts as per the Bureau of Ceylon Standards[6] requirement, the water may be considered suitable to clean processing line, equipment and empty bottles.

TABLE 3. PRESUMPTIVE COLIFORM COUNT AND E. COLI COUNT OF WATER SAMPLES[a]

Tap water sample	No. of tubes positive		Presumptive	
	Mac Conkey broth	2% BGLB and peptone	Coliform count/100ml	*E. coli* count/100ml
1	0	0		
	0	0	Nil	Nil
	0	0		
2	2	0		
	0	0	9	Nil
	0	0		
3	0	0		
	0	0	3	Nil
	1	0		

[a]Average of 20 determinations on different occasions from three water taps.

Enumeration of salmonella: The pasteurized milk in this study did not show characteristic salmonella colonies on selective agars such as bismuth sulphite agar and brilliant green agar.

Sterilization studies of the processing line: Before sterilization (2 per cent sodium hydroxide at 77°C) commenced, pasteurizing vats and homogenizer showed comparatively low levels of total colony counts, while other parts of the processing line showed very high values (often too numerous to count) of total colony counts (Table 4). After sterilization of the processing line, all parts showed a remarkable reduction in total colony count. Swabs taken the next morning, before the pasteurization of milk commenced, showed a slight increase in the total count, probably due to overnight contamination. Despite sterilization, the pipe lines between homogenizer and surface cooler, and from surface cooler and bowl filler and the rubber washers in the bowl filler showed remarkably high total colony counts, compared to other parts of the processing line.

To avoid contamination from the processing line, a mixture of chlorescene (chlorine sanitizer) and soda ash (1:3) should be applied every other day to the bowl filler to cleanse the rubber washers. On alternate days, the vats and the pipe lines must be washed with soap and water and flushed with 200 p.p.m. chlorine. Further sterilization with hot caustic soda solution (2 per cent), followed by sterilization with 0.25 per cent mineral acid solution at 70°C and hot water must be done once a month.

TABLE 4. EFFECT OF STERILIZATION ON THE TOTAL COLONY COUNT AT VARIOUS POINTS[a] OF THE PROCESSING LINE

Source	Total colony count (CFU/ml)×10³		
	Sterilization		Next morning before pasteurization
	Before	After	
Pasteurizing vats			
Vat No. 1	4.3	0.19	0.24
Vat No. 2	5.2	0.23	0.41
Homogenizer	TNTC[b]	0.17	0.28
Condenser between homogenizer and surface cooler	TNTC	0.51	0.62
Pipe line between homogenizer and surface cooler	TNTC	10.6	11.2
Surface cooler	TNTC	0.44	0.52
Top and bottom dishes of *surface cooler*	TNTC	0.47	0.80
Pipe line from cooler to bowl filler	TNTC	7.2	8.1
Bowl filler	TNTC	0.33	0.58
Rubber washers in the bowl filler	TNTC	TNTC	TNTC
Wash bottles stored crater	TNTC	TNTC	TNTC

[a]Estimation on swabs, Mean of 5 estimations on different occasions
[b]TNTC—Too numerous to count

Swabs taken before sterilization from the pasteurizing vats were always free from *E. coli*. In four of the five experiments done on different days of processing, the homogenizer too was free from *E. coli*. Swabs from all other parts of the processing line showed presence of *E. coli* before sterilization. However, they were killed during sterilization.

In the case of crates, washing with hot 2 per cent sodium hydroxide did not reduce the total colony counts, but *E. coli* were destroyed (Table 4). To minimize contamination from the crates, the bottles that are stored in the crates for long periods should be directed again to the washing machine. To prevent overnight contamination, the dairy plant must be kept free from insects, flies, cockroaches and rodents. Good management practices require such pests to be rigidly prevented from entering the premises.

Effectiveness of pasteurization: To ensure proper pasteurization, time during which the milk was held at 62.8°C was checked on several days and found to be always 30 min or longer. Milk was heated to 62.8°C in 40 min, held at this temperature for 30 min, after

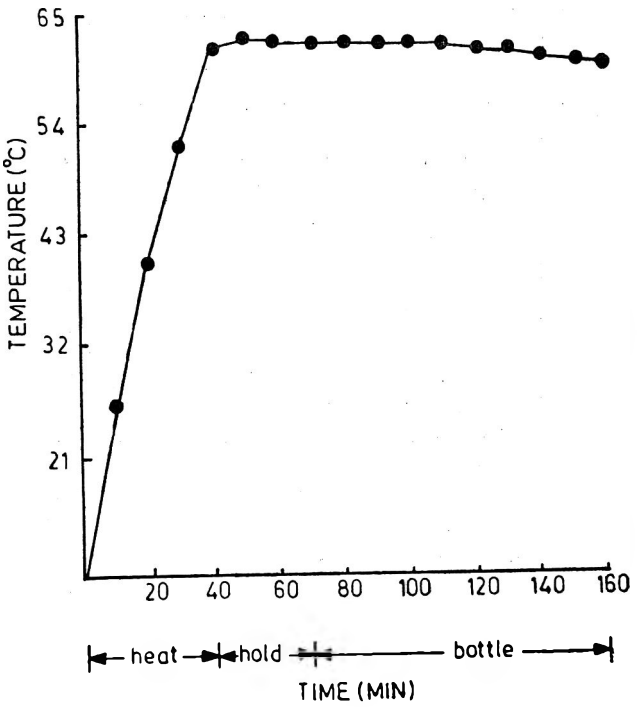

Fig. 1. Time-temperature relationship during milk processing.

which the steam valve was closed to stop heating the milk in the pasteurizing vat and allowed to flow through the processing line. During this period, there was a slight increase of 0.5—1°C in temperature of milk (Fig. 1). However, throughout the period of bottling, the temperature of the milk in the pasteurizing vat remained at 62.8±0.8°C.

Table 5 shows the relationship between time, temperature, total colony count per ml of milk from pasteurizing vat and presence or absence of *E. coli*. Although three separate experiments were performed, the values for total colony count could not be compared, because milk had varying microbial counts on different days. Nevertheless, a general trend was observed. As the time was increased, the number of micro-organisms in the milk from the pasteurizing vat decreased gradually during heating, holding and bottling (Table 5).

In all the experiments, milk was initially contaminated with *E. coli* which was not destroyed during heating. However, they were destroyed during holding period and the milk in the pasteurizing vat remained free from *E. coli* throughout the process of bottling. These results indicate that when properly carried out pasteuri-

TABLE 5. EFFECT OF HEAT TREATMENT (PASTEURIZATION) ON THE TOTAL COLONY COUNT (TCC) AND E. COLI DURING HEATING, HOLDING AND BOTTLING OPERATIONS

Time (min)	Temp. (°C)	First Experiment		Second Experiment		Third Experiment	
		TCC/ml of milk	*E. coli*	TCC/ml of milk	*E. coli*	TCC/ml of milk	*E. coli*
				Heating			
0	0	6.64×10^6	+	1.025×10^7	+	1.32×10^7	+
10	26.1	5.28×10^6	+	5.12×10^6	+	9.21×10^6	+
20	43.0	4.1×10^6	+	1.50×10^6	+	7.23×10^6	+
30	53.5	1.22×10^6	+	1.32×10^6	+	5.19×10^6	+
				Holding			
40	62.8	9.4×10^6	+	1.17×10^6	+	3.19×10^6	+
50	62.8	2.4×10^6	—	2.7×10^5	+	2.81×10^6	+
60	62.8	2.2×10^3	—	2.0×10^5	—	2.06×10^6	—
				Bottling			
70	62.8	2.1×10^5	—	2.1×10^5	—	2.0×10^6	—
80	63.4	1.0×10^5	—	1.8×10^5	—	1.72×10^6	—
90	63.4	1.8×10^5	—	1.5×10^5	—	1.50×10^6	—
100	63.4	8×10^4	—	1.7×10^5	—	1.06×10^6	—
110	63.2	7×10^4	—	1.4×10^5	—	5.6×10^6	—
120	62.8	7×10^4	—	1.0×10^5	—	5.2×10^5	—
130	62.8	6×10^4	—	1.2×10^5	—	4.0×10^5	—
140	62.8	7×10^4	—	8.0×10^4	—	2.8×10^5	—

zation not only reduced the total colony count of micro-organisms but also destroyed *E. coli* completely.

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A Comparison of Physical Quality, Composition, Cholesterol, Vitamin A and Fatty Acid Contents of Guinea Fowl and Chicken Eggs

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Physical quality characteristics, chemical composition, cholesterol, vitamin A and fatty acid composition of Guinea fowl eggs were compared with those of chicken eggs utilizing about 500 eggs from both groups. Besides, acceptability of different egg products prepared from both the groups was compared. Guinea fowl eggs were smaller and had significantly ($P<0.05$) lower albumen and yolk indices, Haugh unit score, per cent albumen but higher yolk colour score, shell thickness, yolk and shell than those of chicken eggs. Total lipid, cholesterol and vitamin A contents were lower in Guinea fowl eggs and practically no difference existed in proximate composition of egg contents and fatty acid contents of yolk lipids. No significant differences were found in various sensory characteristics between different products prepared from the eggs of the 2 species.

Guinea fowl is recently identified for commercial exploitation to meet the growing demand for poultry products in our country. This species has attracted the attention perhaps due to its high adaptability to adverse local conditions and ability to thrive well on forages and insects from the soil under semi-intensive system of management. So far no information is available on physico-chemical characteristics of Guinea fowl eggs. Hence, an attempt was made in this investigation to evaluate the above characteristics and acceptability of Guinea fowl eggs and compare them with those of chicken eggs.

Materials and Methods

Guinea fowl and chicken eggs (500) were obtained from experimental farms of this institute. Eggs were weighed upto 0.01 g and their shape index calculated[1]. Specific gravity[2], albumen index[3] and yolk index[4] of individual eggs were determined. Haugh unit was measured directly by Haughmeter. Yolk colour score was determined by comparing with 'Roche colour fan'. Thickness of shell (without membrane) was measured by Ames thickness measure at three different places and average calculated. Albumen and yolk were separated and weighed. Shell with membrane was dried

at room temperature for 24 hr and weighed. pH and proximate composition[5] of albumen, yolk and egg contents were determined separately on 5 replicates of composite samples from both species. Total lipids, cholesterol and vitamin A of egg yolk were determined according to procedures of Bligh and Dyer[6], Wootton[7] and Carr and Price[8] respectively. Fatty acids were determined after preparation of methyl esters[9], by gas liquid chromatography (Chemito 3800 of Toshniwal Instruments, Bombay) using flame ionization detector and 8 per cent diethylene glycol succinate (DEGS) column, having oven, injection and detection temperatures of 200 , 220 and 230°C respectively.

Sensory evaluation of different egg products viz, hard boiled, poached, scrambled and omelette, was conducted to evaluate colour, texture, chewability, flavour and over-all acceptability using routine score sheet. Statistical analysis[10] of data was done following standard procedure. Wherever means were found significant, Duncan's multiple range test[11] was applied.

Results and Discussion

Table 1 represents the physical quality characteristics of Guinea fowl and chicken eggs. Guinea fowl eggs had significantly ($P < 0.05$) lower weight (38.04 g), albumen and yolk indices and Haugh unit score and higher yolk colour score and shell thickness than those of chicken, while shape index and specific gravity were similar in both the species. Egg weight and shell thickness reported earlier[12-15] agreed well with the present findings. Proportion of various egg components (Table 1) revealed a significantly lower value for the per cent albumen and absolute weight of albumen and yolk in Guinea fowl egg but a higher percentage of yolk than those of chicken. Per cent albumen as reported[13,14] also closely agreed with the present value. Yolk: albumen ratio of guinea fowl egg was also significantly higher than chicken egg. However, absolute and relative weights of shell (per cent) were higher in Guinea fowl eggs than the chicken eggs indicating that Guinea fowl eggs had a thicker and stronger shell. Similar findings had been reported earlier[14,15].

Chicken eggs contained more total lipid, cholesterol and vitamin A than Guinea fowl eggs but differences in cholesterol concentration (per g yolk) were insignificant (Table 2). The higher content of the above constituents may be attributed to the bigger yolk size of chicken egg. No significant difference was obtained

TABLE 1. PHYSICAL QUALITY AND COMPONENT PARTS OF GUINEA FOWL AND CHICKEN EGGS

Parameters	Guinea fowl	Chicken
Egg wt (g)	$38.04^a \pm 0.24$	$51.25^b \pm 0.30$
Shape index	$78.37^a \pm 0.45$	$75.36^a \pm 0.22$
Albumen index	$0.084^a \pm 0.000$	$0.094^b \pm 0.001$
Yolk index	$0.382^a \pm 0.000$	$0.438^b \pm 0.002$
Haugh unit	$78.91^a \pm 0.68$	$68.88^b \pm 0.43$
Yolk colour score	$10.58^a \pm 0.75$	$7.83^b \pm 0.10$
Shell thickness (mm)	$0.45^a \pm 0.00$	$0.33^b \pm 0.00$
Specific gravity	$1.084^a \pm 0.374$	$1.097^a \pm 0.001$
Albumen wt (g)	$18.32^a \pm 0.14$	$29.86^b \pm 0.22$
Yolk wt (g)	$12.08^a \pm 0.09$	$14.87^b \pm 0.12$
Yolk: Albumen ratio	$0.66^a \pm 0.01$	$0.50^b \pm 0.00$
Shell with membranes wt (g)	$5.50^a \pm 0.06$	$5.19^b \pm 0.04$
% Albumen wt	$48.20^a \pm 0.24$	$58.29^b \pm 0.29$
% Yolk wt	$31.85^a \pm 0.22$	$29.07^b \pm 0.22$
% Shell with membranes wt	$14.45^a \pm 0.13$	$10.16^b \pm 0.09$

Means with same superscript in each raw are not significantly ($P<0.05$) different.

TABLE 2. TOTAL LIPID, TOTAL CHOLESTEROL, VITAMIN A AND FATTY ACID CONTENTS OF GUINEA FOWL AND CHICKEN EGGS

Parameters	Guinea fowl	Chicken
Content/g yolk		
Total lipid (mg)	$352.0^a \pm 8.2$	$417.7^b \pm 21.7$
Total cholesterol (mg)	$3.5^a \pm 0.2$	$4.0^a \pm 0.4$
Vitamin A (I.U.)	$16.4^a \pm 0.5$	$22.4^b \pm 1.3$
Content in one egg		
Total lipid (g)	$4.3^a \pm 0.2$	$6.2^b \pm 0.3$
Total cholesterol (mg)	$41.9^a \pm 2.4$	$59.8^b \pm 5.5$
Vitamin A (I.U.)	$198.5^a \pm 6.2$	$333.6^b \pm 19.7$
Fatty acids (%)		
Palmitic acid	$28.0^a \pm 1.0$	$28.7^a \pm 2.2$
Palmi-toleic acid	$7.8^a \pm 1.4$	$4.8^a \pm 1.6$
Stearic acid	$10.8^a \pm 1.1$	$8.0^a \pm 0.9$
Oleic acid	$33.3^a \pm 1.2$	$47.5^b \pm 1.5$
Linoleic acid	$12.1^a \pm 0.8$	$10.0^a \pm 0.2$
Arachidic acid	$3.4^a \pm 0.6$	$1.2^a \pm 0.2$

(N = 6)
Means with same superscript in each row are not significantly ($P<0.05$) different

TABLE 3. PROXIMATE COMPOSITION AND pH OF EGG COMPONENTS OF GUINEA FOWL AND CHICKEN

Constituents	Egg contents		Albumen		Yolk	
	Guinea fowl	Chicken	Guinea fowl	Chicken	Guinea fowl	Chicken
Moisture (%)	67.7[a] ±1.3	69.1[a] ±0.6	87.7[a] ±0.3	89.3[b] ±0.1	47.5[a] ±0.3	45.0[b] ±0.7
Total protein (%)	14.4[a] ±0.3	13.9[a] ±1.0	10.4[a] ±0.3	8.2[b] ±0.1	17.8[a] ±0.3	15.8[b] ±0.2
Ether extract (%)	13.1[a] ±0.2	12.8[a] ±0.9	0.2[a] ±0.1	0.2[a] ±0.1	31.8[a] ±0.3	41.0[b] ±1.4
Total ash (%)	1.2[a] ±0.1	0.9[b] ±0.02	0.6[a] ±0.0	0.5[a] ±0.02	1.7[a] ±0.0	1.9[a] ±0.03
pH	7.2[a] ±0.0	7.5[b] ±0.04	9.0[a] ±0.0	8.4[b] ±0.1	5.9[a] ±0.0	6.3[b] ±0.03

(n = 6)
Means with same superscript in each row are not significantly ($P < 0.05$) different.

in fatty acid contents of yolk except oleic acid which was higher in chicken eggs than Guinea fowl eggs (Table 2). Comparison of proximate composition (Table 3) of egg contents did not show any significant differences between species in any of the nutrients except total ash which was significantly higher in Guinea fowl eggs. Per cent moisture and total protein content of albumen were lower and higher respectively in Guinea fowl eggs than chicken eggs but ether extract and total ash content of albumen were statistically non-significant. Conversely, Guinea fowl yolk had higher moisture and total protein content and lower ether extract than its counterpart while total ash content was similar in both species. Similar findings[16] were also observed in proximate composition. Lower pH of egg contents and yolk and higher pH of albumen was obtained in Guinea fowl eggs than chicken eggs.

Sensory evaluation of different egg products like hard-boiled, poached, scrambled and omelette prepared from Guinea fowl and chicken eggs did not

TABLE 4. SENSORY SCORES* FOR DIFFERENT PRODUCTS PREPARED FROM GUINEA FOWL (GF) AND CHICKEN EGGS (C)

Sensory characteristics	Scores for different products							
	Boiled egg		Poached egg		Scrambled egg		Omelette	
	C	GF	C	GF	C	GF	C	GF
Colour	3.17[a] ±0.17	3.83[a] ±0.17	3.67[a] ±0.20	4.00[a] ±0.00	3.67[a] ±0.20	3.83[a] ±0.17	3.17[a] ±0.17	3.33[a] ±0.20
Texture	3.83[a] ±0.17	3.83[a] ±0.17	3.50[a] ±0.35	3.83[a] ±0.17	3.33[a] ±0.20	3.33[a] ±0.20	3.83[a] ±0.17	3.67[a] ±0.20
Chewability	4.00[a] ±0.00	3.83[a] ±0.17	3.33[a] ±0.20	3.83[a] ±0.17	3.33[a] ±0.20	3.83[a] ±0.17	3.83[a] ±0.17	3.67[a] ±0.20
Flavour	3.83[a] ±0.17	4.00[a] ±0.00	3.33[a] ±0.20	3.67[a] ±0.20	3.50[a] ±0.35	3.50[a] ±0.59	3.67[a] ±0.20	3.83[a] ±0.17
Overall acceptability	5.82[a] ±0.17	6.50[b] ±0.22	5.50[a] ±0.62	6.00[a] ±0.26	5.50[a] ±0.22	5.53[a] ±0.71	6.00[a] ±0.00	6.17[a] ±0.36

* The sensory score varies from 1 (very poor) to 4 (very good) for colour, texture, chewability, flavour and from 1 (dislike extremely) to 7 (like extremely) for overall acceptability, respectively. Means with same superscript in a row product-wise did not differ significantly ($P < 0.05$).

differ significantly for any of the attributes. (Table 4). However, hard boiled Guinea fowl eggs were significantly superior to chicken eggs in colour and over-all acceptability.

From this study, it can be concluded that Guinea fowl eggs are as good as chicken eggs in quality and acceptability. Rather, it has the added advantage of having a thicker shell which can with stand the rough handling better during transport and marketing.

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Measurement of Denaturation of Fish, Goat and Beef Proteins—A Viscometric Study with Protein Stabilised Emulsions

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Thermal transition of fish, goat and beef proteins have been determined from the protein stabilised food emulsions using a rotating viscometer. The emulsions have been characterised by non-Newtonian index STI (Shear thining or thickening index). With clear protein solutions, Ostwald viscometry has been applied for determination of transition temperature. The emulsion method is found to give same results as the Ostwald's method. This is also confirmed from an experiment with a standard protein bovine serum albumin. Transition temperatures of fish, goat and beef proteins have been determined at different pH values. Transition temperature of fish myosin was also determined both by Ostwald and rotating viscometer. The data have been utilised to calculate quantitatively the fraction of protein denatured at any temperature. The advantage of the emulsion method over the existing methods has been discussed.

The toughening of muscle during frozen storage is a rheological change. The alteration in texture increases as the cold storage period progresses[1]. Muscle texture can either be measured directly by studying the rheological properties or indirectly by observing some physical and chemical parameters related to texture. In the study of rheological properties, the most widely used techniques are based on compressive and penetrative, shear and creep properties of the muscle[2-5]. These tend to give results that are neither very reliable nor strictly reproducible.

The direct techniques include cell fragility tests[1], changes in protein solubility[6] and water binding capacity[7]. These methods utilise the change in a particular property of the protein as a measure of the extent of denaturation and thus do not measure the absolute value of denaturation.

Transition temperatures of biopolymers in presence and absence of salt have been measured by polarimetry[8], colorimetry[9], PH-metry[10], viscometry[11] and other physico-chemical techniques[12]. Viscometry has been applied to determine protein denaturation in muscle systems[13-15]. In the present paper, thermal transition of fish, goat and beef proteins have been studied by viscometry.

Materials and Methods

Raw materials: Fresh water fish Catla (*catla catla* average wt. 1-1.5 kg) was procured from the market. Flesh free from skin and bone was separated from the dorsal part and used. Goat meat and beef procured from the local market were deboned before use. Salt used was of 'pro-analysi' grade and solutions were made in double distilled water. Double refined peanut oil was procured from the market and used.

Preparation of salt soluble protein extract: One hundred grams of meat from goat, beef or fish were mixed and blended with 650 ml of cold (5-6°C) 0.5 M NaCl solution in an electric blender for 5 min. The slurry was cooled to 2-3°C and centrifuged at 5000 rpm for 10 min in a table top centrifuge. The centrifugate was stored at 5°C and used for emulsification as salt soluble protein extract.

Preparation of fish myosin: Fish myosin was prepared following the method of Connell[16].

Emulsion preparation: For each experiment, 150 g of muscle was blended with 650 ml of NaCl solution (0.5M); 10 per cent oil (65ml) was then slowly added and emulsified. This was taken in stoppered bottles and calculated amount of salt was added and mixed thoroughly to get definite concentration of those salts.

Adjustment of pH: Hydrochloric acid or caustic soda solution was used to adjust the pH. After initial adjustment, the emulsions were stored in a refrigerator (5°C) for 16 hr. The emulsions were then brought to room temperature and the pH was measured again before studying the effect of temperature.

Viscosity measurement by Brookfield viscometer: Apparent non-Newtonian viscosity of the emulsion was measured in a Brookfield synchro-lectic viscometer (model LVT) for characterisation of the non-Newtonian behaviour, measurements being carried out at different rpm settings. When the viscosity of the system was very small as in the case of BSA or myosin solution, measurements were carried out in a UL-adopter supplied with the instrument. For transition temperature measurements, the same emulsion was equilibrated at each temperature for about 15 min and then readings were taken.

Determination of transition temperature by Ostwald viscometry: The Ostwald viscometric method for determination of transition temperature was essentially the same as described by Bull[9]; temperature was controlled to ± 0.1°C. Transition profile was obtained by plotting $\eta sp/C$ against temperature. Flow times used for calculation of ηsp were the average of three replicates. The reproducibility of flow time was very high, being within ± 0.2 sec.

Determination of iso-electric point of salt extracted fish protein: The isoelectric pH of absorbed fish protein was determined by the micro-electrophoresis method. A little groundnut oil was emulsified in 0.02 M NaCl extracted fish protein and the emulsion droplets taken in a rectangular electrophoretic cell was focussed through a microscope fitted with a micrometer scale in the eyepiece. The scale was placed horizontally and the mobility of the oil droplets having the adsorbed protein films was measured by a stop watch after application of appropriate potential gradient. The detailed procedure of measurement was reported elsewhere[17], pH of the emulsion was adjusted prior to the experiment by suitable addition of acid or alkali. Several velocity measurements were averaged and the polarity was reversed for each determination. The electrophoretic mobility was calculated using Smoluchowski equation.

$$\delta = \frac{4\pi\eta U}{D} \times (300)^2$$

Where η and D are the velocity and dielectric constant of the medium which is taken as the same as that of water at 25°C.

Results and Discussion

Characterisation of emulsions: Emulsion viscosity is often very complex and difficult to analyse. This is primarily because of the inability in predicting the exact hydrodynamic conditions because of non-Newtonian behaviour. However, Rosen[19] has described a simple and useful method of characterising these emulsions. The method is, however, applicable to fluids obeying power law. Analysis of the data for the fish slurry emulsion indicated that it obeys the power law (Dial reading=K (rpm)^n; k and n being constants). Rosen's method consists of assigning an index called shear thining index (STI) (>1.0) or shear thickening index (STI) (<1.0), as the case may be. The shear thining or thickening index (STI) values, which are

TABLE 1. SHEAR THINNING INDEX VALUES FOR FISH EMULSION AT DIFFERENT pH VALUES

pH=4.64		pH=8.04		pH=5.72	
Temp. (°C)	Shear thinning index	Temp. (°C)	Shear thinning index	Temp. (°C)	Shear thinning index
24.0	2.04±0.06*	24.9	2.23±0.07	25.1	4.42±0.21
27.2	2.26±0.08	28.0	1.91±0.02	27.5	3.29±0.17
30.2	2.26±0.04	31.0	1.68±0.10	30.6	2.77±0.08
32.6	2.83±0.09	33.1	1.86±0.09	32.4	2.30±0.11
34.8	2.38±0.05	35.1	2.12±0.07	34.1	2.20±0.13
37.6	2.00±0.06	37.5	3.20±0.16	36.1	2.00±0.12

*Standard error.

TABLE 2. SHEAR THINNING INDEX VALUES FOR BEEF EMULSION AT DIFFERENT pH VALUES

pH=3.55		pH=5.43		pH=7.50	
Temp. (°C)	Shear thinning index	Temp. (°C)	Shear thinning index	Temp. (°C)	Shear thinning index
23.2	1.16±0.06*	21.9	2.81±0.17	22.4	2.03±0.06
27.5	1.20±0.05	25.0	2.89±0.18	28.4	2.36±0.12
31.5	1.25±0.10	28.2	3.11±0.21	33.6	2.35±0.20
35.5	1.26±0.08	31.0	3.08±0.11	35.6	2.39±0.08
39.4	1.31±0.10	34.4	3.03±0.11	37.8	2.91±0.10
40.6	1.43±0.11	36.4	3.04±0.09	41.6	3.38±0.13
42.0	1.62±0.07	38.6	2.72±0.07	45.9	3.51±0.12
43.4	1.82±0.09	41.6	3.22±0.12		
47.7	2.30±0.14	44.6	3.57±0.14		

*Standard error.

TABLE 3. SHEAR THINNING VALUES FOR GOAT MEAT EMULSION AT DIFFERENT pH VALUES

pH = 3.95		pH = 8.05		pH = 5.95	
Temp. (°C)	Shear thinning index	Temp. (°C)	Shear thinning index	Temp. (°C)	Shear thinning index
23.5	1.49±0.05	22.5	3.26±0.12	23.6	7.10±0.91
29.2	1.62±0.04	28.8	3.21±0.16	30.0	7.75±0.48
34.2	2.02±0.08	34.2	3.00±0.21	35.0	8.08±0.57
37.2	2.41±0.21	37.0	2.52±0.19	37.4	8.15±0.82
39.8	2.70±0.26	39.8	2.26±0.09	39.8	6.62±0.65
41.4	2.48±0.17	42.8	1.91±0.16	41.3	5.53±0.31
44.0	2.89±0.18	46.0	2.48±0.11	42.8	4.13±0.21
47.8	3.04±0.19	48.0	6.92±0.48	44.3	3.19±0.14
52.2	3.25±0.20			45.8	2.69±0.16
56.8	2.94±0.16			49.7	11.16±0.88

measures of deviation from Newtonian behaviour, are reported for all the emulsions studied (Tables 1, 2 and 3). The emulsions show shear thining behaviour as all the STI values were greater than 1.0.

Thermal transition of salt extracted fish protein by Ostwald-viscometer: The specific viscosity (ηsp) at each temperature was determined and $\eta sp/C$ decreased steadily until around 35°C it rose sharply indicating a transition temperature of 35°C for the salt extracted fish protein (Table 4). Such rise in $\eta sp/C$ is well established for globular proteins[20]. It may be noted that these viscosity values are apparent and relative and in no way represent Tanford's value of globular proteins or randon coils. Fig 1 shows the variation of torque as a function of temperature for fish slurry (0.5 M NaCl) emulsion. The results showed that there is a gradual fall in torque upto about 34°C, above this temperature there is a sharp increase showing a transition temperature of 34°C. The same trend was observed for rpm settings of 6, 12, 30 and 60 indicating the reproducibility of the measurements. Above 36°C, the emulsion became very much unstable with immediate formation of cream and subsequent coalescence. So, measurements at higher temperatures were not possible.

This inference was confirmed by similar studies on the behaviour of 9 per cent BSA (0.1 M NaCl, pH 6.50) and 1.8 per cent BSA stabilised emulsion (0.1 M NaCl, pH 6.50, $\Phi = 0.44$). The different viscosity behaviour of emulsion and solution near the transition temperature (sharp rise in torque for emulsion and sharp fall for solution) may be due to different mechanism. In emulsion, a BSA molecule with its globular

TABLE 4. TRANSITION TEMPERATURE OF DIFFERENT PROTEINS DETERMINED BY APPARENT VISCOSITY MEASUREMENT OF PROTEIN SOLUTIONS AND PROTEIN STABILISED EMULSIONS

System studied	Ionic strength M(NaCl)	pH	Viscometer used	Transition temp (°C)
Fish salt extract	0.5	5.72	Ostwald	35.0
Fish meat emulsion	0.5	6.50	Brookfield	34.0
BSA solution	0.1	6.50	Brookfield	62.0
BSA emulsion	0.1	6.50	Brookfield	62.0
Fish myosin solution	0.5	5.60	Ostwald	38.0
Fish myosin solution	0.5	5.60	Brookfield	39.0
Goat meat emulsion	0.5	8.05	Brookfield	42.0
Goat meat emulsion	0.5	5.95	Brookfield	42.0
Goat meat emulsion	0.5	3.95	Brookfield	42.0
Beef meat emulsion	0.5	7.00	Brookfield	36.5
Beef meat emulsion	0.5	5.43	Brookfield	34.0
Beef meat emulsion	0.5	3.62	Brookfield	Transition not clear
Fish meat emulsion	0.5	8.04	Brookfield	36.5
Fish meat emulsion	0.5	5.72	Brookfield	35.0
Fish meat emulsion	0.5	3.92	Brookfield	Transition not clear
Beef salt extract	0.5	5.70	Ostwald	37.0
Goat protein salt extract	0.5	5.70	Ostwald	43.0

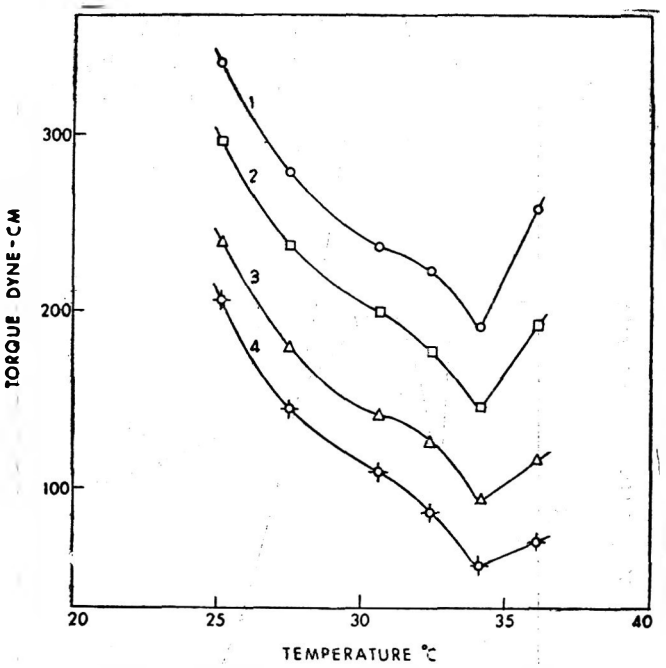

Fig. 1. Plot of torque against temperature for 10% peanut oil emulsion in fish slurry at different rpm.

NaCl concentration is 0.5 M. 1:60 rpm, 2:30 rpm, 3:12 rpm, 4:6 rpm.

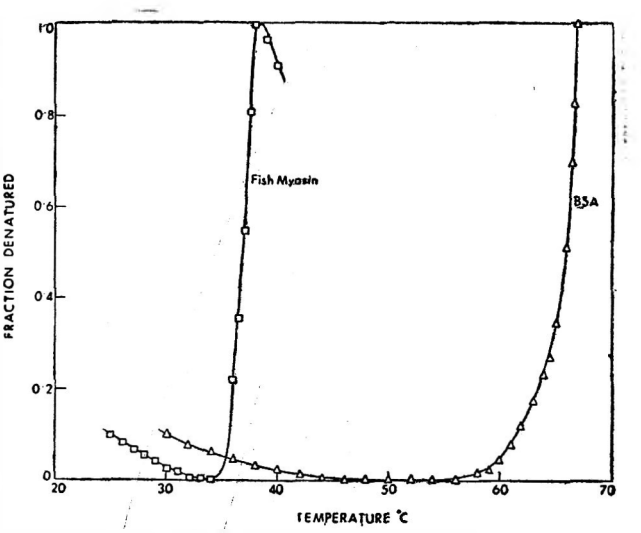

Fig. 2. Plot of fraction denatured against temperature for 1.8% BSA emulsion and 5% myosin solution.

Ionic strength BSA = 0.1 (NaCl), Myosin = 0.5 (NaCl), pH: BSA = 6.50, Myosin = 5.60.

structure can be attached to one droplet only. But around the transition temperature, when the chains are opening up, the extended segments can get themselves attached to several other droplets. Thus, a network of entrapped droplets may be set up which can account for the increased viscosity. This type of loop interaction mechanism leading to a flocculated system was suggested earlier by Das and Chattoraj[21] for BSA stabilised emulsion.

The transition temperature for fish myosin was 38°C as determined by Ostwald viscometer, whereas it was 39°C as determined by using the Brookfield viscometer (Table 4).

Quantification of thermal transition: Thermal transition of protein can be quantified by defining ft, the transition fraction in thermal denaturation.

$$ft = \frac{q_t - q_o}{\Delta q_{max}}$$

Where q is a property such as viscosity of the protein system[22]. The subscripts 't' and 'o' refer to an experiment at an elevated temperature 't' and a reference temperature at which q is temperature independent and ft=0 respectively. Δq_{max} is given by—

$$\Delta q_{max} = q_{max} - q_o$$

Where q_{max} represents the maximum value of q[23]. The temperature at which ft becomes 0.5 has been called the denaturation temperature. The temperature range at which ft changes from 0.25 to 0.75 is called the transition half-width[23]. Fig 2 shows a typical plot of transition fraction of myosin and BSA as a function of temperature using Brookfield apparent viscosity data. It is seen that the thermal transition of fish myosin is very sharp, the complete transition occurring in 2-3°C temperature interval. For BSA, although initially transition is gradual, the transition half width is about 2°C indicating sharp temperature transition. So, denaturation temperature can be determined unambiguously.

From microelectrophoretic studies of adsorbed fish proteins on peanut oil, the adsorbed protein has been found to have an iso-electric pH very close to 5.2. As can be seen from Fig 3 the viscosity values rises steeply at pH 5.2, reaches a maximum around pH 6.3 and then falls. This result implies that the properties of the emulsion reflects the properties of the protein which stabilises it.

The effect of the concentration of two denaturing agents, urea and potassium thiocyanate, (KSCN) on a emulsion of 10 per cent by volume of peanut oil stabilised by fish protein has been shown in Fig 4. At low concentration, KSCN is known to have a stabilising effect on protein structure[23]. With the salt concentration, viscosity rises first, reaches a maximum and then decreases. The curve for urea also follows the same pattern. Such denaturing agents which exert their effects through the modification of the structure of water can be classed as hydrophobic bond breaking agents. Detailed mechanism of their denaturing effects are not yet established. This is primarily because the structure of water in concentrated electrolyte solution is highly complex and for that the previous speculations about the function of water in stabilising the secondary

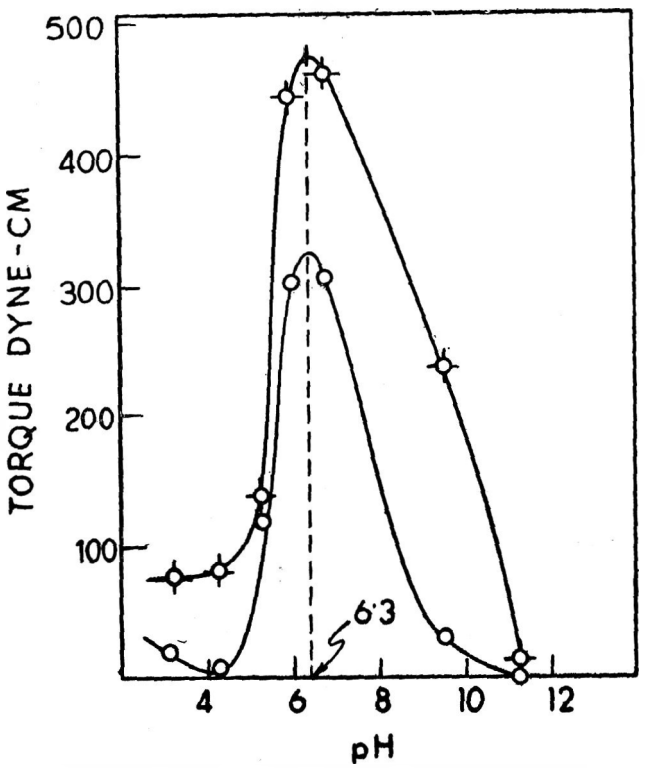

Fig. 3. Variation of torque as a function of pH of emulsion of 10% peanut oil in fish slurry. NaCl concentration in aqueous medium is 0.5 M, 60 rpm, 0, 6 rpm.

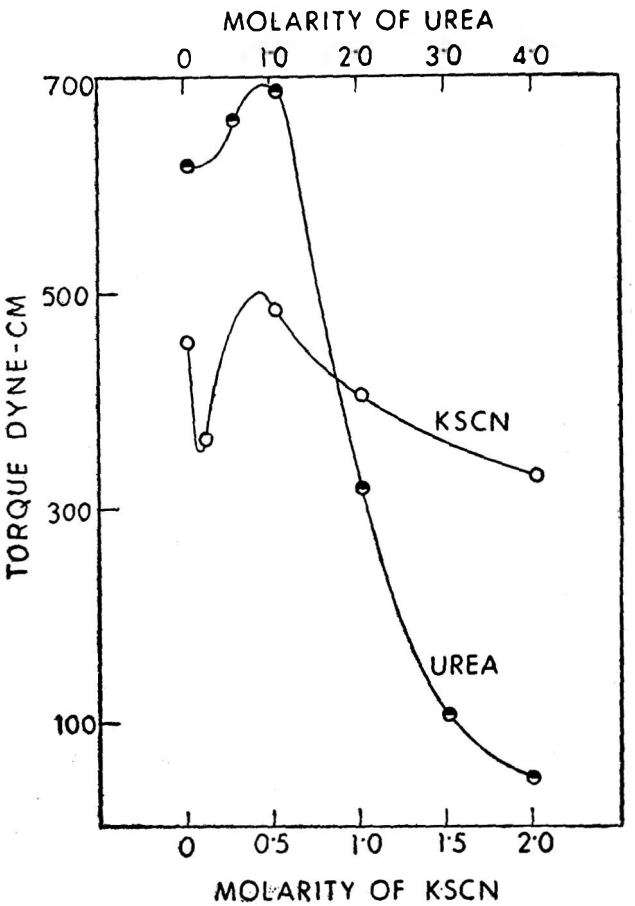

Fig. 4. Plot of torque against molarity of denaturants for fish slurry emulsion of 10% peanut oil, Ionic strength = 0.5 (NaCl), pH = 6.50.

structure of proteins have been proved to be more speculative than accurate[24,25].

Thermal transition of goat and beef proteins: Studies have also been extended with goat and beef proteins to get information about their thermal denaturation characteristics in emulsion systems. The results at different pH values are compared with fish emulsions. All these emulsions are characterised in terms of their deviations from Newtonian behaviour by finding out the STI values at different pH. The values are presented in Tables 1, 2 and 3. Goat meat emulsions at pH 8.05, 5.95 and 3.95 showed same denaturation temperature of 42°C. Emulsions stabilised by beef proteins showed denaturation temperature of 36.5°C and 34°C at pH 7.0 and 5.43 respectively and that stabilised by fish protein 36.5°C and 35°C at pH 8.04 and 5.72 respectively. The change in viscosity with temperature in the acidic pH for all the three emulsions are not very conspicuous. A visible change of curdling was observed in all the cases. Probably, denaturation was effected at the very beginning by acid itself. Alkaline pH did not cause predenaturation and hence it was possible to identify denaturation temperature in the thermal study. The transition temperature for goat and beef proteins were also determined by Ostwald's viscometric method using the 0.5 M salt extract at pH 5.70. The values of 43°C and 37°C for goat and beef proteins respectively are close to those obtained by the emulsion method.

From the above discussion, it is clear that the thermal transition properties of the proteins in solutions can be reproduced satisfactorily with emulsions stabilised by same proteins. Emulsion method has got several advantages for the study of thermal transition of fish and other animal proteins over studies with protein isolate. Preparation of emulsion is easier than protein isolation involving several steps. Emulsion technique is free from the problem of quick sedimentation which makes the study with protein isolates often in the form of protein slurry or suspension quite difficult. Consistency is better reproducible with an emulsion. Emulsions have a far greater viscosity than the corresponding protein isolate so that available commercial instruments can adequately determine the changes due to denaturation whereas with protein isolate, precision is needed for determining thermal changes which are usually small.

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Studies on Transportation of Wet Fish. IV. Use of Liquid Nitrogen as a Secondary Refrigerant

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Fresh fish can be transported in 4 in. polyurethane foam insulated chamber along with ice (25% of the weight of fish) at a temperature of 5°C with the help of evaporating liquid nitrogen (LN). A 25% increase in LN consumption over usual requirement for heat leakage i.e. 0.004 kgLN/hr/m²/°C $\triangle$T is expected if used in this operation.

In our earlier papers[1-3] the situation in our country in respect of fresh fish transport has been reported and possible ways to overcome it were also suggested. Substantial work has been conducted at Central Institute of Fisheries Technology, Cochin, along with other collaborating organisations to find out a solution to the problem of transportation of wet fish under extreme ambient conditions prevailing in this country[4-7]. As fish is transported with ice in varied proportions depending on the climatic conditions in non-insulated railway vans and trucks, use of returnable G.I. containers with expanded polystyrene insulation was suggested[8]. The use of such a returnable container involves high initial investment, labour charge for cleaning, return freight charge and the possibility of its loss during the return trip.

The present study was undertaken to find out the possibility of using a secondary refrigeration system like evaporating liquid nitrogen without disturbing the present mode of handling fresh fish.

Materials and Methods

Model insulated unit: A laboratory model insulated unit was fabricated from aluminium sheet (2 mm) with 4 in. polyurethane foam slab insulation for simulated transportation studies. The unit has an outside surface area 4.2 sq. m.

Maintenance of temperature: A steady state temperature of 4°C was maintained inside the insulated chamber by allowing liquid nitrogen (LN) to evaporate from an insulated container with an exposed area of evaporation of 0.07 sq.m. The inside temperature of the chamber was brought down to a steady state temperature of 4°C from an initial temperature of 33°C within 130 min from the time of initial exposure.

Temperature measurement: The temperature at different points inside the chamber was monitored through a multipoint digital temperature indicator (Laxons, Bombay) using platinum resistance temperature sensors (Pt-100 ohm at 0°C).

LN consumption: LN consumption was estimated by direct weighing of the LN-container at different time intervals.

Ice melting: The ice melting under various conditions was estimated from the volume of ice-melt water collected at different time intervals.

Fish: Labeo rohita (average wt. 1 kg), the most popular variety of carp being transported from different states to Calcutta, the major fish consuming centre, was selected. The fish temperature was measured as per the procedure of Graham[9].

Ice: Potable water—ice prepared in the laboratory was used. The crushed ice had a bulk density of 600 kg/cu. m.

Results and Discussion

Fig 1 shows the cooling of a 0.23 cu. m. capacity insulated chamber using ice and liquid nitrogen (LN) as refrigerant. The drop in the inside temperature is rapid during the first one hour of cooling operation, $\triangle$T=24° for LN and 14° for ice. Liquid nitrogen as a fluid with high heat transfer coefficient requires com-

*Address of correspondence.

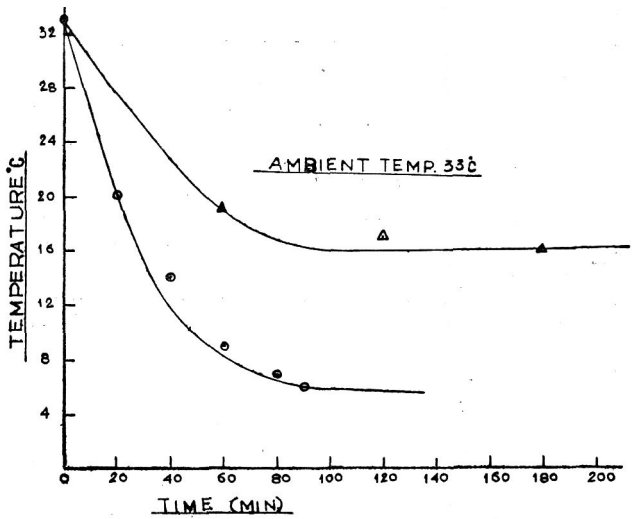

Fig. 1. Cooling of a 0.23 cu.m. polyurethane (4 in) insulated chamber with ice and liquid nitrogen.

—△—△— 0.33 sq.m. exposed surface ice —⊙—⊙— 0.07 sq.m. exposed surface liquid nitrogen (LN)

paratively less heat transfer surface i. e. 0.07 sq. m. than solid ice of 0.96 sq. m. for same refrigeration effect. In other words, keeping the bulk density of ice same, half of the insulated space would be occupied by ice to have similar refrigeration effect as liquid nitrogen.

This reduces the pay load for ice-fish transport even when the chamber is insulated with 4 in. polyurethane foam. As an alternative, a liquid nitrogen evaporating system requiring comparatively little space can be accommodated inside the insulated chamber without significant effect on pay load. This is the basis of a commercial system known as "Pelican" (British Oxygen Co., U.K.). Such a system automatically attains a steady state temperature with the help of evaporating LN depending on available heat transfer surface for the evaporating liquid and the thermophysical properties of LN-container material.

Fresh fish should be transported at a temperature of 50°C[4,5]. It is possible to bring down the inside temperature of insulated chamber to around 5°C within a period of two hr by the use of evaporating LN but the fish temperature cannot be brought down so quickly with cold nitrogen gas due to the poor heat transfer between stagnant nitrogen gas and fish (Fig 2). Moreover, this will lead to surface dessication of fish with unacceptable appearance. In the case of combination refrigeration, where fish covered with crushed ice is allowed to cool in an environment maintained at 4°C with evaporating liquid nitrogen in an insulated chamber fish temperature was found to drop to about 4°C within a period of an hr which would have dropped to 9°C

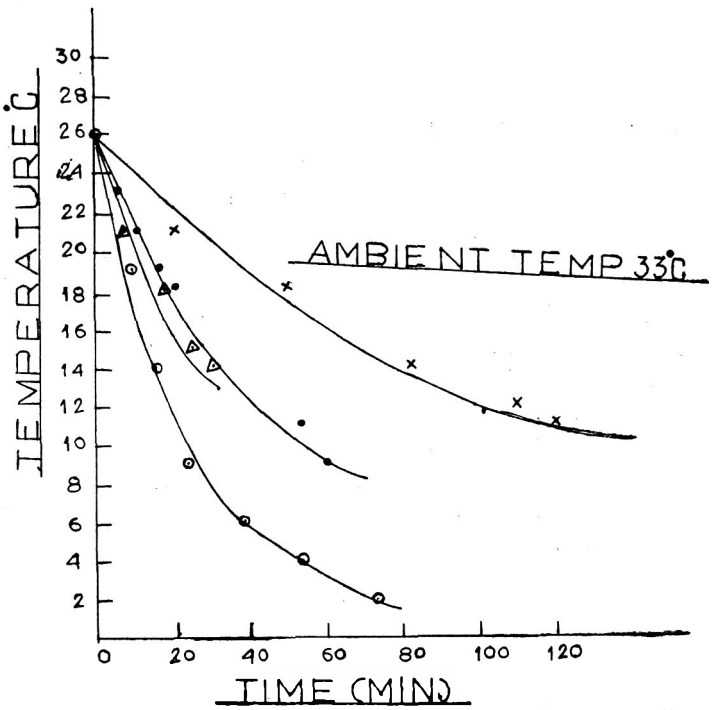

Fig. 2. Time-temperature profile for fish *Labeo rohita* (Rohu) during chilling with different refrigerants.

—⊙—⊙— ice and LN; —×—×—LN; —.—.— ice; —△—△— ice and fish in open atmosphere.

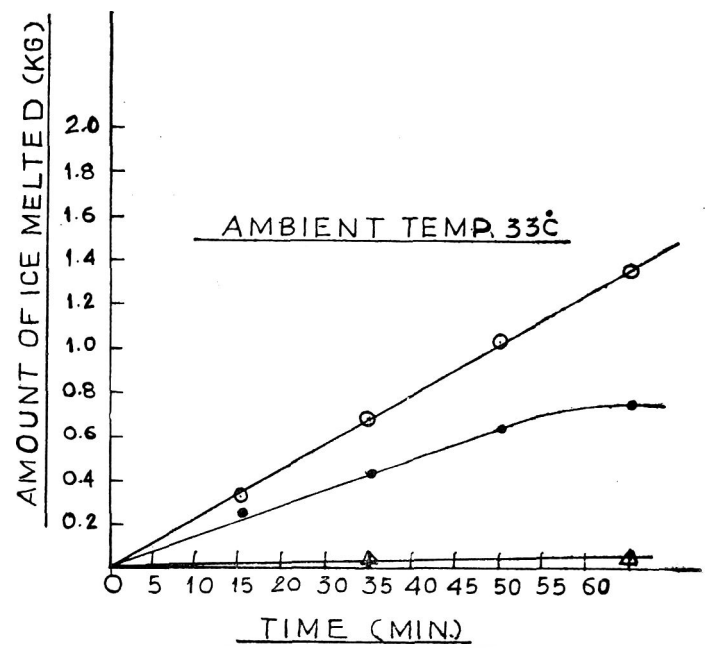

Fig. 3. Ice melting in different transport environments

—⊙—⊙— ice in open atmosphere; —.—.— ice in 4" polyurethane foam insulated chamber; —△—△— ice in 4" polyurethane foam insulated chamber and maintained at 4°C by evaporating LN.

with only ice. However, in the former, even the fish is completely covered with crushed ice, a 25 per cent increase in liquid nitrogen consumption was noticed apart from liquid nitrogen requirement for heat leakage (0.004 kg LN/hr/m²/°CΔT) into the system. This is due to the fact that cold nitrogen gas penetrates through crushed ice and comes in contact with fish already at a higher temperature. Ice melting will be reduced in this case and only the quantity of ice which is necessary to cool down fish from ambient to 5°C is required to be used and is optimum, which is about 25 per cent of the weight of fish.

Fig 3 indicates that the ice melting rate can be reduced by about 50% using an enclosure with 4 in. polyurethane foam insulation and this can be further reduced to a negligible amount (0.05 kg/hr) if a secondary refrigerant like evaporating LN is used to maintain a temperature around 4°C within the enclosure.

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Effect of Processing on Protein Quality and Mimosine Content of Soo-Babul *(Leucaena leucocephala)*

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The kernel of Soo-babul (*Leucaena leucocephala*) seed was found to have high percentage of protein (52.51%), calcium (0.57%) and phosphorus (0.48%). However, it contained a high level (12.72%) of the toxic amino acid, mimosine which vitiated the protein quality of the seed. When the seed was soaked, dehusked, pressure cooked and washed for 72 hr in running water, mimosine content got reduced to 0.27%. Processed seed fed to rats showed insignificant toxic effects whereas unprocessed seeds led to severe harmful effects such as hair and weight loss, swelling of liver, kidney, etc. and also death in some cases.

Leucaena Leucocephala, commonly known as Soo-babul in India, has excellent forage qualities. It is highly productive and readily consumed by livestock. The leaves, young stems, flowers and pods are good sources of protein and minerals[1]. They have good amino acid availability and are rich in β-carotene and other vitamins and serve as exceptional sources of calcium and phosphorus[2,3]. However a major constraint in the use of the plant as animal feed is the presence of the toxic amino acid, 'mimosine' [β-N (3 hydroxy-4-Oxopyridyl) α-amino propionic acid]. This toxin occurs in seeds as also in all other parts of the plant[2]. The toxic effects of feeding *Leucaena* to ruminants and non-ruminants were reported by several workers[1,3-6].

In Central America and Indonesia, young leaves, small pods and roasted seeds are used as human food. No toxic effects were, however, reported in these countries possibly because the consumption was too low to have any harmful effects[2]. Earlier investigations showed that heating *Leucaena* in a closed atmosphere[7], air-drying at elevated temperatures[4] or heat treatment of

leaves and seeds after moistening[7] resulted in lowering the mimosine content. Addition of small amounts of soluble iron salts to the feed was reported to counteract the toxic effects to a considerable degree[8].

The present study was taken up to see if mimosine could be eliminated by subjecting *Leucaena* seeds to suitable treatments before using them for human consumption and also to evaluate the effect of such treatments on the protein quality.

Materials and Methods

Leucaena seeds, procured in Andhra Pradesh, were chemically analysed by the AOAC[9] methods to assess the nutrient composition. The seeds were subjected to the following treatments:

1. Seeds were dehusked using a PRL mini-dehuller and (*a*) powdered as such or (*b*) roasted at 110°C and then powdered.
2. Seeds were pressure cooked at 15 psi, dehusked manually, oven dried at 60°C and powdered.
3. Seeds were soaked overnight in water, coat removed manually and (*a*) kept as they were, or (*b*) cooked in an equal amount of water and washed 4 times in tap water; or (*c*) pressure cooked at 15 psi for 30 min and washed in running tap water for 72 hr. Each was then oven dried and powdered.

Mimosine was determined by the colorimetric method[10]. Tannin content was estimated by the modified vanillin hydrochloride method[11]. Trypsin inhibitors were estimated by Kakade's method[12]. The amino acid composition of the whole seed was determined after hydrolysis using Technicon Auto Analyser (Beckman model 119 CL). The diets for the biological experiments were prepared using processed and unprocessed *Leucaena* seeds as source of protein at 10 per cent level; the diet included groundnut oil (9 per cent), vitamin mixture (2 per cent), mineral mixture (4 per cent) and cellulose (4 per cent). Rest of the feed was made up of corn starch.

Male weanling (21 to 23 day old) albino rats were used for the study; they were divided into seven groups of eight each and were placed in individual cages. Records of food consumption and weight gain were maintained. PER was determined at the end of 4 weeks by the method of Osborne *et al.*[13] The clinical symptoms observed were also regularly recorded. At the end of the experiment, thyroid, liver, heart, kidney and testes of the rats were histologically examined[14].

Results and Discussion

The nutrient composition of *Leucaena* seed with and without seed coat is given in Table 1. The kernel had high protein content (52.51 per cent). Calcium and phosphorus contents were also high. As shown in Table 2, the essential amino acid content of the seed compared favourably with that of the reference protein.

Table 1. CHEMICAL COMPOSITION OF LEUCAENA SEEDS

Constituents	With seed coat (%)	Without seed coat (%)
Moisture	9.30	8.60
Fat	7.50	15.50
Protein	32.90	52.50
Ash	0.78	1.91
Calcium	0.42	0.57
Phosphorus	0.64	0.48
Iron	0.014	0.025

Values are means of three trials.

Table 2. AMINO ACID COMPOSITION (G PER 100 G PROTEIN) OF LEUCAENA SEEDS

Amino acids	Leucaena	FAO Reference Protein*
Aspartic acid	8.98	7.7
Threonine	2.26	4.0
Serine	3.36	7.7
Glutamic acid	11.35	14.7
Proline	2.78	10.7
Glycine	3.68	2.2
Alanine	3.00	6.1
Cystine/Methionine	0.87/0.85	3.5
Valine	3.02	5.0
Isoleucine	15.88	4.0
Leucine	4.84	7.4
Tryosine	2.42	3.0
Phenylalanine	3.02	3.0
Histidine	2.53	2.5
Lysine	4.21	5.4
Arginine	6.17	5.2

*FAO/WHO provisional pattern 1973 values for Leucaena are means of two trials.

The mimosine level was as high as 12.72 per cent in raw kernel (Table 3) which vitiated its protein quality. Simple overnight soaking of the seed or roasting of raw kernel had no effect on the level of mimosine but pressure cooking followed by dehusking reduced it to 4.77 per cent. More elaborate treatment reduced the level further. The best method was found to be soaking followed by dehusking manually, pressure cooking and finally washing for 72 hr in running water, which reduced the level to 0.27 per cent.

Other antinutritional factors like trypsin inhibitor and tannin were found to be negligible.

Rats fed treated seeds showed a higher PER than those which received unprocessed seed. The PER improved as the seed was processed further (Table 3). The seed given maximum treatment showed a PER

TABLE 3. EFFECT OF DIFFERENT TREATMENTS ON MIMOSINE AND PER OF LEUCAENA SEEDS AND CASEIN

Group	Protein source	Mimosine (g%)	PER
A	Casein	—	3.02
B	Raw kernel with coat	8.00	—
	without coat	12.72	-2.01
C	Soaked and dehusked	12.00	-1.02
D	Raw kernel roasted	12.40	-0.99
E	Pressure cooked without washing and dehusked	4.77	+0.58
F	Soaked, dehusked, cooked and washed 4 times	1.29	+1.00
G	Soaked, dehusked, pressure cooked (30 min) and washed (72 hr) in running water	0.27	+1.99

Experiment with Groups B, C, D, E and F was terminated after 2/3 weeks; Groups A and G were continued for 4 weeks.

of 1.99, which compared well with that reported for other legumes like Bengal gram (1.83), lentil (1.93), black gram (1.52) and green gram (1.72)[15].

The histological studies showed maximum toxic effects in rats (Group B) fed raw untreated kernel. On the other hand, those fed diet containing processed seeds having only 0.27 per cent of mimosine (Group G) showed the least toxic effects. In other groups, the toxic symptoms were somewhat proportionate to the mimosine content of the diet. Rats which were fed raw kernel showed significant weight and hair loss and severe deficiency symptoms such as opacity of lens and cornea and exopthalmas. The liver evidenced cloudy swelling and fatty degenerative changes indicative of toxic damage. In kidney, the changes were more pronounced starting from cloudy swelling of tubules and terminating in necrosis of particularly proximal convoluted tubules. The thyroid showed contracted acini while the testes evidenced less number of spermatids and spermatocytes indicative of the effect of the toxin on reproductive organs. Also, two of the rats of this group died on the 18th day of the experiment.

Rats (Group G) which received processed seed with only 0.27 per cent mimosine showed normal weight gain. However, loss of hair on the sides of the abdomen was noticed even in this group after 3 weeks of feeding. The liver showed mild cloudy swelling indicating minimal damage to liver parenchyma. Kidney showed no change while the thyroid showed distention of acini indicating mild goitre.

It was thus seen that even after elaborate treatment, the seed still contained a certain percentage of the toxin though to a very small extent. The PER improved but the toxic effect though negligible, was not desirable.

Further studies are needed to find out easier methods to eliminate the mimosine level completely from the *Leucaena* seed so that it could safely be used as animal feed or for human consumption for prolonged periods.

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RESEARCH NOTES

EFFECT OF FORMALIN ON THE GERBER FAT TEST

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The Gerber method gave consistently low fat values in milk samples preserved with formalin. The fat values decreased with the increase of formalin concentration and the hydrometer reading [due to higher SNF or proteins] of milk samples. The storage of the preserved milk samples further affected the Gerber fat results. Rose-Gottlieb method also gave slightly low fat values in milk samples containing 0.4% formalin.

In our country, use of formalin at 0.4 per cent level has been prescribed for preservation of milk samples under the Prevention of Food Adulteration Act (PFA), 1954. However, information on the effect of formalin on milk fat determination by standard Gerber method is rather contradictory. While Sandhu, *et al.*[1] observed no significant change in the fat content of milk samples preserved with 0.4 per cent formalin even when stored upto 12 months, other workers[2-5] have claimed that Gerber method gives low fat values in milk samples preserved with formalin. Several queries, regarding the effect of formalin on the Gerber fat test, have been received from various organized dairies when their milk samples were found substandard under the PFA. In view of these, a detailed study on the effect of formalin on Gerber fat test was undertaken.

Composite buffalo and cow's milk samples were collected from the Experimental Dairy of the National Dairy Research Institute, Karnal. The buffalo and cow's milk samples (standardized to 7 per cent and 4 per cent fat and 22° to 32° hydrometer readings with skim milk powder, cream and water) were treated with formalin (37-40 per cent formaldehyde, Glaxo Laboratories) at various concentrations (0.1-0.4 ml per 100 ml milk). The samples were analysed for fat by the Gerber[6] and Rose-Gottlieb[7] methods before and after treatment and storage at various temperatures. In the Gerber method, the butyrometers were shaken vigorously till the visible flakes/clots of coagulated milk proteins disappeared. Formalin treated and stored samples were invariably given more vigorous shaking.

It is evident from the results presented in Table 1 that the Gerber fat values went on decreasing with the increase of hydrometer reading and formalin concentration in the milk samples. Formalin at 0.1, 0.2, 0.3 and 0.4 per cent did not affect the Gerber fat values of the milk samples having hydrometer readings upto 27, 25, 23 and 22°C, respectively. Thereafter, the fat values decreased with the increase of hydrometer reading. At 32° hydrometer reading, the fat values were 0.5, 1.2, 2.0 and 3.0 per cent less in milk samples containing 0.1, 0.2, 0.3 and 0.4 per cent formalin, respectively. Similar effect was observed in cow's milk samples when treated with 0.4 per cent formalin. During storage, the fat values further decreased with the increase in storage period. The Gerber fat readings of milk samples (treated with 0.4 per cent formalin) having 23 and 26° hydrometer readings were 6.95 and 6.50 per cent on 0 day which decreased to 6.45 and 6.00 respectively on 30th day of storage.

TABLE 1. EFFECT OF FORMALIN ON PER CENT FAT (GERBER TEST) IN BUFFALO MILK

Hydrometer reading (30°C)	Control	Preserved with formalin			
		0.1%	0.2%	0.3%	0.4%
22	7.00	7.00	7.00	7.00	7.00
23	7.00	7.00	7.00	7.00	6.95
24	7.05	7.05	7.05	7.00	6.95
25	7.00	7.00	7.00	6.95	6.70
26	6.95	6.95	6.90	6.80	6.45
27	7.00	7.00	6.90	6.75	6.20
28	7.05	7.00	6.80	6.45	5.95
29	7.00	6.90	6.65	6.10	5.50
30	7.05	6.85	6.45	5.80	5.15
31	7.05	6.75	6.20	5.45	4.65
32	7.00	6.50	5.80	5.00	4.00

Low fat exhibited by the Gerber method in the milk samples treated with formalin may be due to incomplete dissolution of milk proteins, as formaldehyde is known to harden the proteins. Higher the concentration of formalin in milk and higher the hydrometer reading (higher SNF or proteins), greater was the difficulty experienced in dissolving the butyrometer contents during shaking. The proteins did not dissolve completely but dispersed in fine particles, which were deposited below the fat layer after centrifugation. Some

TABLE 2. EFFECT OF 0.4 PER CENT FORMALIN ON MILK FAT PER CENT AS DETERMINED BY ROSE-GOTTLIEB METHOD AFTER 30 DAYS

Hydrometer reading (30°C)	Milk fat (%)		
	Control	0-day	30th day
23.0	7.00	6.95	6.92
24.0	4.16	4.04	4.00
25.0	4.15	4.12	3.85
25.7	6.70	6.61	6.52
25.9	6.99	6.96	6.87
25.9	7.05	6.96	6.80
26.0	7.01	6.91	6.83
26.1	6.88	6.68	6.63
26.2	6.73	6.60	6.58
26.5	6.86	6.80	6.73
26.8	6.13	6.01	5.93

of the fat either in free or globular form, might have remained entrapped in the undissolved protein layer and this may have resulted in low fat values by the Gerber method. Other workers[2-5] also reported low fat values by Gerber method in milk samples preserved with formalin. Pien *et al*[3] even suggested that formalin treated milk samples should not be analysed by Gerber method. However, Sandhu, *et al.*[1] did not find significant change in the fat values of cow's milk samples having 0.4 per cent formalin, even during storage upto 12 months.

The Rose-Gottlieb method also gave low fat values in the milk samples treated with formalin at 0.4 per cent concentration. The fat values were 0.02 to 0.21 and 0.13 to 0.30 per cent less than those of control samples, on 0 day and 30th day of storage respectively (Table 2). Bector and Narayanan[8], however, reported no change in fat values of preserved milk samples by Rose-Gottlieb method. The slight decrease in fat values by Rose-Gottlieb method in formalin treated milk samples may also be due to incomplete dissolution of proteins with ammonia, which was evident from the turbidity in the fat extraction tubes.

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CHEMICAL AND BIOCHEMICAL INVESTIGATIONS ON NORMAL, CHALKY AND MODIFIED OPAQUE-2 STRAINS OF MAIZE

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Protein, lysine and tryptophan contents of Opaque-2 varieties as a group were significantly higher than in the normal type. Differences between chalky and hard-endosperm type with regard to protein and tryptophan contents were not significant; however, it was significant for lysine. Similar trend was also found for chemical score. On an average, total starch content in hard endosperm type was comparable to the normal endosperm types and was distinctly higher than in the chalky types. *In vitro* protein digestibility for hard endosperm opaque-2 [88.3%] was intermediate between the chalky type [90.5%] and normal types [87.3%].

Improvement of the protein quality of maize (*Zea mays* L.) kernels by using opaque-2 gene has been underway under the aegis of All India Co-ordinated

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Maize Improvement Project (AICMIP)[1-3]. Soft nature of the endosperm has been found to be largely responsible for lack of extensive acceptability of opaque-2 maize varieties. Incorporation of opaque-2 in normal maize varieties is known to favourably modify the amino acid composition of the endosperm primarily due to reduction in the zein fraction of protein which is largely deficient in lysine and tryptophan[4]. In the present study, protein qualities of some of the hard endosperm types of 'opaque-2' maize, were compared with the normal and chalky opaque-2 varieties.

Some of the promising hard-endosperm strains of opaque-2 selected from SO/SN composite for kernel vitreosity were compared with chalky opaque-2 (Shakti' and 'Rattan') and normal maize hybrids '(Ganga 5' and 'Deccan 103') forming ten treatments in all. Protein, ash, fat and crude fibre were determined according to the recommended AOAC methods[5] and starch determined by the polarimetric method[6]. Lysine and tryptophan in defatted samples were estimated by colorimetric method described by Villegas and Mertz[7] and Hernandez and Bates[8], respectively. *In vitro* protein digestibility (IVPD) was determined by the method of Saunders and Kohler[9] as modified by Gupta *et al.*[10] The data were analysed statistically as completely randomised design with two replications according to the method suggested by Panse and Sukhatme[11].

Protein and starch contents ranged from 9.85 to 12.91 and 64.33 to 70.57 per cent, while the ranges in values of fat, ash and crude fibre contents were 4.33 to 5.89, 1.35 to 1.92 and 2.18 to 2.91 per cent, respectively (Table 1). Protein to starch ratio on an average was

TABLE 1. KERNEL WEIGHT, CHEMICAL COMPOSITION AND PROTEIN-STARCH RATIO OF SOME MAIZE VARIETIES (% DRY BASIS)

Variety/ Pedigree*	100 kernel wt (g)	Protein (%)	Starch (%)	Fat (%)	Ash (%)	Crude fibre (%)	Protein/ starch ratio
Modified opaque-2							
1.	18.94	12.54	69.38	4.40	1.35	2.61	0.18
2.	22.98	11.64	68.39	4.9_	1.92	2.55	0.17
3.	18.01	11.75	66.97	4.33	1.83	2.22	0.18
4.	17.14	12.91	64.38	4.68	1.85	2.18	0.20
5.	20.17	12.86	69.90	4.35	1.90	2.74	0.18
6.	18.84	12.30	64.38	5.05	1.60	2.91	0.19
Mean	19.35	12.33	67.23	4.63	1.74	2.53	0.18
Chalky opaque-2							
7.	24.47	12.83	66.88	5.15	1.87	2.60	0.19
8.	22.56	11.78	64.33	5.51	1.67	2.74	0.18
Mean	23.52	12.30	65.60	5.35	1.77	2.67	0.19
Normal							
9.	31.11	11.11	70.57	4.43	1.40	2.76	0.16
10.	30.40	9.85	65.05	5.89	1.69	2.83	0.15
Mean	30.76	10.48	67.81	5.16	1.55	2.80	0.16
CD at 5%	3.69	0.55	4.38	0.51	0.18	0.39	
S.E.M. (at 9 df)	±1.15	±0.17	±1.37	±0.16	±0.05	±0.12	

*1. SN composite (Bulk)≠(SN≠)$_5$ SN
2. SN composite ≠(SN≠)$_8$ SN
3. SO/SN/Composite (Bulk) FS≠SN
4. SO/SN composite (E)≠ABP≠(SN≠) FS-1, 50% FS-8≠ FS-3.
5. SO/SN composite (F)≠(SN≠) 5-SN^2-FS-2.
6. SO/SN composite (Reconstituted)≠SN
7. Shakti.
8. Rattan.
9. Ganga-5
10. Deccan 103

higher in chalky opaque-2 and hard endosperm opaque-2 varieties than in the normal maize genotypes. The hard endosperm selections, however, showed marked variations in protein and starch contents suggesting the possibility of combining these two traits at various levels.

The lysine and tryptophan contents, on an average, in hard endosperm opaque-2 were 29.4 and 71.4 per cent respectively and in chalky opaque-2 51.8 and 81.0 per cent higher than the normal maize hybrids (Table 2). These changes are significant at 5 per cent level. Similar trend was also apparent for chemical score. *In vitro* protein digestibility, which determines the bioavailability of food proteins[12], was higher for opaque-2.

In most of the opaque-2 maize breeding programmes, tryptophan or lysine contents have been used as an index for identifying strains with better protein quality. Data for these two amino acids in the hard endosperm selection showed marked variations. Selection high in lysine and tryptophan resulted in high chemical score. Selections made for hard endosperm also varied in proteins, starch yield and several other agronomic traits. Several of these characters appear to be independent in inheritance. Any breeding programme designed at improving the kernel vitreosity should include comprehensive evaluation for various chemical traits. Such an approach would yield strains which are superior in agronomic traits as well as protein quality. The number of selections which are to be handled in the laboratory, can be substantially reduced by restricting the evaluation to only the agronomically superior materials.

TABLE 2. LYSINE, TRYPTOPHAN AND IN VITRO PROTEIN DIGESTIBILITY (PD) OF SOME MAIZE VARIETIES

Variety*	Lysine (g/16 N)	Tryptophan (g/16g N)	*In vitro* PD (%)	Chemical score (%)
Modified opaque-2				
1.	3.87	1.15	79.94	70.36
2.	4.85	1.15	88.47	88.18
3.	3.18	1.09	84.28	57.82
4.	4.26	1.13	85.06	77.45
5.	3.61	0.95	93.37	65.64
6.	3.43	1.01	88.42	62.36
Mean	3.87	1.08	88.26	70.30
Chalky opaque-2				
7.	4.13	1.13	89.88	75.09
8.	4.95	1.15	91.16	90.00
Mean	4.54	1.14	90.52	82.55
Normal				
9.	2.99	0.68	87.34	54.36
10.	2.98	0.58	87.32	54.18
Mean	2.99	0.63	87.33	54.27
CD at 5%	0.32	0.10	1.19	
S.E.M. (at 9 df)	±0.10	±0.03	±0.50	

*For Pedigree *See* Table 1

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EFFECT OF AFLATOXIN ON SEED GERMINATION OF BENGAL GRAM (*CICER ARIETINUM*)

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The effect of aflatoxin on Bengal gram seed germination and hypocotyle elongation was studied at 0, 20, 40, 60, 80 and 100 ml/μg concentration of aflatoxin. With increase in aflatoxin concentration, seed germination was inhibited whereas hypocotyle elongation was unaffected.

Aflatoxin, a secondary metabolite of *Aspergillus flavus*, was found to reduce the per cent seed germination in many crops. Pulses are more susceptible to mold attack and toxin production. A considerable reduction of germination in cress (*Lepidium sativum*) by crude aflatoxin at 2.5 μg/ml was observed[1]. Inhibition of seed germination by aflatoxin was reported in cowpea[2], groundnut, green gram, black gram[3], maize[4] and also in soybean[5]. The reduction in elongation and dry weight of roots and stems of groundnut seedlings by aflatoxin treatment was reported[6]. In the present investigation, an attempt was made to study the effect of various levels of aflatoxin on seed germination and hypocotyle elongation of Bengal gram.

Toxic effect of aflatoxin on seed germination was tested on Bengal gram of local variety and 'Annigeri-1' seeds. The seeds were surface sterilized with 0.1 per cent mercuric chloride solution for 2 min and then washed thoroughly with sterile distilled water. These surface sterilized seeds were soaked in 0,20,40,60,80 and 100 μg/ml concentration of aflatoxin for 12 hr. Surface sterilized seeds soaked in sterile distilled water for the same period served as controls. The seeds were taken out after 12 hr, washed, blot dried and placed on sterile moist filter paper in sterilized germinating discs (100 seeds/disc) and were incubated at room temperature (28±2°C). The filter paper was kept moist. Four replications were maintained for each treatment. The percentage of seed germination and hypocotyle elongation were scored on the seventh day.

The results indicated a higher germination in 'Annigeri-1' compared to local variety. The inhibition of germination significantly increased with increase in the concentration of aflatoxin in both the varieties. However, the inhibitory effect was slightly higher in the variety 'Annigeri-1' than local variety (Table 1).

TABLE 1. EFFECT OF AFLATOXIN ON GERMINATION AND AND HYPOCOTYLE ELONGATION OF BENGAL GRAM SEEDS

Aflatoxin concn. (μg/ml)	Local variety		Annigeri-1	
	% germination	Hypocotyle elongation (cm)	% germination	Hypocotyle elongation (cm)
0	81.0 (—)	2.0	89.0 (—)	2.0
20	78.0 (3.7)	2.0	82.0 (7.9)	1.9
40	70.5 (12.9)	1.9	74.0 (16.8)	2.0
60	65.0 (19.8)	1.9	70.0 (21.4)	2.0
80	62.5 (22.8)	1.9	68.0 (23.6)	1.9
100	60.5 (25.3)	2.0	65.5 (26.4)	1.9
CD at P=0.05	3.87		4.37	

Figures in parentheses indicate % inhibition.

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HISTOCHEMICAL CHANGES IN THE COTYLEDONS OF *CICER ARIETINUM* SEEDLINGS RAISED FROM AFLATOXIN TREATED SEEDS

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Histochemical changes in the cotyledons of normal and aflatoxin B_1 treated seedlings of Bengal gram [*Cicer arietinum*] were studied. From third day of germination starch and protein degradation began in the untreated cotyledons [controls] whereas they remained clumped in those of the cotyledons of the aflatoxin treated seeds. Further, it was observed that aflatoxin inhibited amylase activity in the treated cotyledons of Bengal gram seeds.

Aflatoxin, a secondary matabolite of *Aspergillus flavus*, has been known to inhibit seed germination in various pulses. It is reported to interfere with the synthesis of proteins by inhibiting enzyme activities[1] and uptake and incorporation of amino acids into proteins[2]. A very slow degradation of reserve proteins in aflatoxin treated cotyledons of cucumber was noticed by Singh and Sharma[3]. Mishra and Tripathi[4] reported that the respiration and alpha-amylase activity were inhibited by aflatoxin in maize. The present investigation was aimed to find out the histochemical changes and amylase activity in the cotyledons of Bengal gram treated with aflatoxin.

Histochemical studies: The seeds of Bengal gram (*Cicer arietinum*) were surface sterilized with 0.1 per cent mercuric chloride for 2 min followed by rinsing with distilled water. The surface sterilized seeds were soaked in aflatoxin B_1 at 100 μg/ml concentration and incubated at 4°C for 12 hr, seeds soaked in sterile distilled water served as control. The seeds were then sown in pots containing washed, sterilized sand and allowed to germinate in the green house. The cotyledons were taken out at one-day intervals upto 7 days and fixed in Cornoy's B fixative (6 parts ethyl alcohol +3 parts of chloroform +1 part of acetic acid) for 2 hr and dehydrated using alcohol-butanol grades viz 50,60,70, 80,90 per cent and absolute alcohol followed by absolute alcohol-butanol grades in 3:1, 1:1 and 1:3 proportions respectively and finally with pure butanol twice. The dehydrated cotyledons were embedded in paraffin wax and 8 μm thick sections were taken using Erma rotary microtome. The sections of both aflatoxin treated and control cotyledons were subjected to histochemical examination viz. periodic acid Schiff's (PAS) test for insoluble polysaccharides[5] and mercuric bromo-phenol blue (MBPB) test for insoluble proteins[6] and were observed under Carl'Zeiss microscope with photomicrographic attachment.

Amylase activity: The cotyledons of germinating control and aflatoxin treated, Bengal gram seeds were taken at one-day intervals (as and when they were taken for histochemical investigation) and amylase extract was prepared by grinding 0.5 g of cotyledon in 10 ml of 0.1M phosphate buffer. Then, the suspended portion was separated by centrifugation at 5000 $\times$ g for 10 min.

Buffered starch solution of 0.3 per cent was pipetted out into test tubes (0.5 ml/tube), were pre-incubated at 37°C for 5 min and 0.6 ml of the enzyme extract was added. The reaction was stopped at the end of 0,6,12,18 and 24 min using 3,5—dinitrosalicylic acid reagent and an aliquot was diluted with 18 ml of distilled water. The absorbance was recorded at 540 nm in a Spectronic-20 spectrophotometer and amylase activity was determined by calculating the amount of glucose released at different intervals using a standard curve for glucose.

TABLE 1. AMOUNT OF GLUCOSE RELEASED AT DIFFERENT PERIODS IN CONTROL (C) AND AFLATOXIN TREATED (T) COTYLEDONS (μG OF GLUCOSE/ML OF STARCH)

Incubation period	1st day		2nd day		3rd day		4th day		5th day		6th day		7th day	
(min)	C	T	C	T	C	T	C	T	C	T	C	T	C	T
0	3.6	3.5	3.0	3.2	3.5	3.5	3.6	3.4	3.7	3.6	2.2	2.2	2.4	2.7
6	4.7	4.5	6.0	4.3	4.5	6.2	6.0	4.5	4.7	4.7	4.7	2.7	4.8	3.3
12	6.0	5.5	6.7	5.2	6.8	6.7	6.6	5.3	6.2	5.1	6.0	3.7	6.7	3.8
18	8.2	7.4	7.6	6.8	7.6	7.0	7.8	6.2	6.7	6.0	6.9	4.6	7.6	3.9
24	9.5	8.7	9.8	8.7	9.3	7.4	9.5	7.6	8.7	6.8	9.5	4.6	9.8	4.7

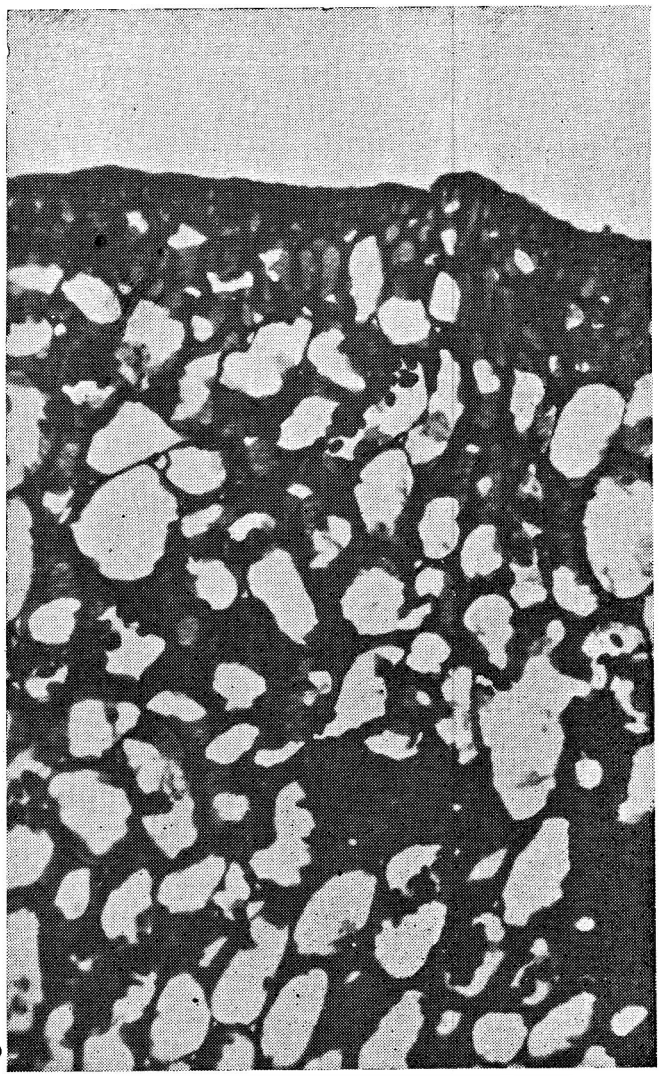

Fig. 1. Section from 7-day old seedlings showing the degradation of stored starch.

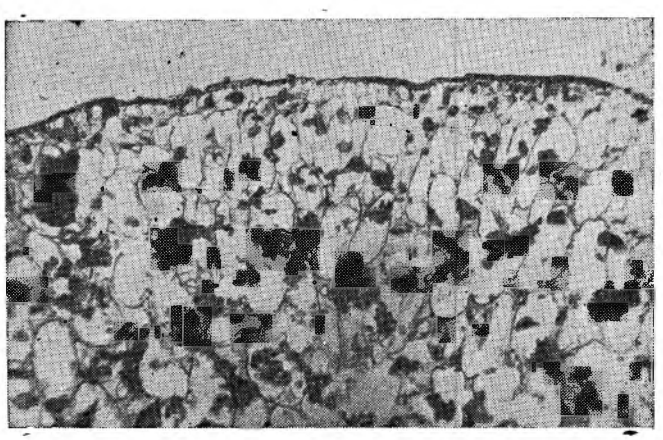

Fig. 2. Section from 7-day old seedling showing the degradation of stored proteins.

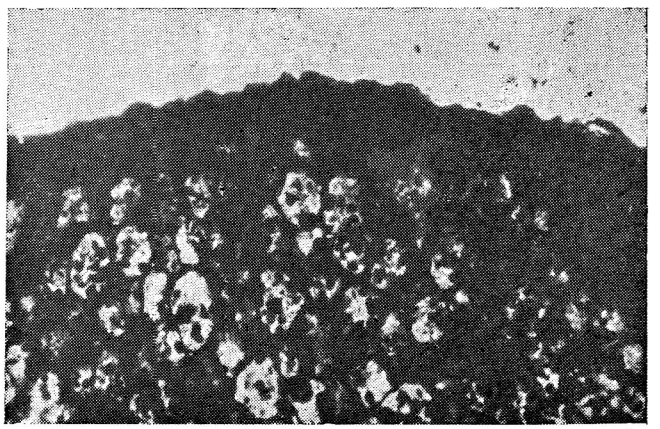

Fig. 3. Section of 7-day old aflatoxin-treated seeds tested with PAS showing clumping of starch grains.

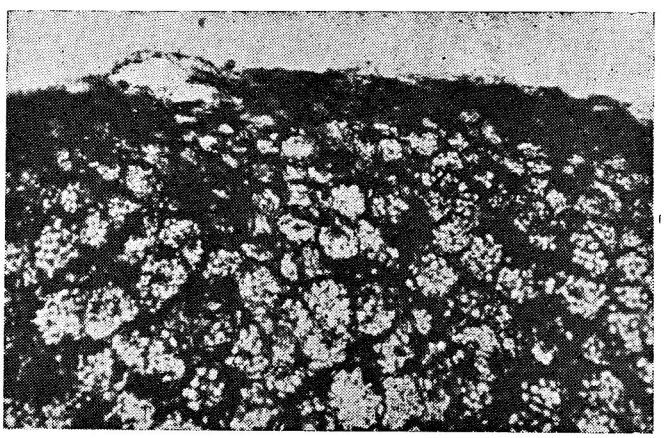

Fig. 4. Section of 7-day old aflatoxin-treated seeds tested with MBPB showing clumping of protein granules.

The normal degradation of reserve starch and proteins started from the third day and by seventh day both were almost completely exhausted in the cotyledons of control (Fig. 1 and Fig. 2). Whereas, they remained almost undiminished but clumped (Fig. 3 and Fig. 4) in those of the treated cotyledons.

There was not much difference in the amount of glucose released per ml of buffered starch solution in control and treated cotyledons at the initial stages. But after 3 days, there was reduction in the amount of glucose released in the control (Table 1) and the same effect continued upto 7 days.

Absence of degradation of starch in the treated seeds was perhaps due to the inhibition of the amylase activity which is responsible for the conversion of starch into metabolically utilizable simpler carbohydrates[1,4]. Similarly, aflatoxin also inhibited the protease activity which was essential for the degradation of proteins into polypeptides and amino acids[2,3,7].

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PREPARATION AND NUTRITIVE VALUE OF PROTEIN ISOLATE FROM COTTONSEED FLOUR

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Protein isolate was prepared from solvent extracted cottonseed flour by conventional alkali peptization method. The extractability of the protein was 73% by the two-stage extraction procedure. The protein isolate had low free gossypol content [<0.06%] with a protein efficiency ratio of 2.1 and available lysine content of 3.3–3.5 g/16 g N. Colour improved on washing the flour prior to extraction but extractability of protein was reduced to 52%. Further improvement of colour [light yellowish] was achieved by hydrogen peroxide treatment. It was essential to supplement with lysine and methionine to improve the nutritive value of the peroxide treated protein.

Technological developments have resulted in the production of edible quality cottonseed flour containing protein of high nutritive value with low free and total gossypol contents[1–4]. The edible flour could be used in food formulations for enrichment of low cost cereal mixtures. However, for the preparation of speciality foods, it is essential to use the protein isolate. It has been reported earlier from this laboratory that by alkali peptization method, it was possible to extract protein, practically free from gossypol from solvent extracted cottonseed meal[5]. This paper reports results of the studies carried out on the extraction efficiency, improvement of colour and the nutritive value of the isolate prepared by alkali extraction procedure.

Dehulled and delinted cottonseed kernels obtained from an oil factory were flaked to 0.2 mm thickness in a Malmo Flaker, extracted with n-hexane at room temperature (25°C) and the residual solvent was removed by exposure to sunlight. The desolventized flakes were ground to a mesh size of 80-100. In some experiments, flakes were used as such.

A few batches (2 kg) were run by adopting conventional alkali peptization method. The extraction was carried out at pH 8.5 by maintaining a flour to water ratio at 1:10. After extraction for a specified time (30-60 min), the dispersion was centrifuged in a solid bowl centrifuge (Bird centrifuge) and clarified further in a high speed Sharples centrifuge (12,000 rpm) to remove starch and fine particles present in the peptized liquor. The protein was preciptated from the liquor at the isoelectric pH (4.8).

To improve the extraction efficiency and colour of the protein, the flakes were soaked and washed continuously by spraying water on a Sharples Vibro Screen. The purple coloured liquor was discarded and the washed material passed through a Fryma emery grinder. After adjusting the pH to 10.0, the slurry was stirred for 30 min and centrifuged to separate protein. For improvement of colour of the protein, hydrogen peroxide (w/v 20 volumes) was added to the peptized liquor (20 ml/l) and the mixture was heated at 80°C for 20 min before precipitating protein at pH 4.8.

Protein content in the isolate was estimated by the micro-Kjeldhal method. Free and total gossypol contents were estimated by the Pon's method[6,7]. Available lysine in the protein was determined by Carpenter's method[8]. Nutritive value of the protein isolate was assessed by the protein efficiency ratio method[9].

It has been shown earlier that the extraction of free gossypol from gland rich solvent extracted meal decreased as the pH increased[5]. Hence, this conventional alkali extraction method was adopted for the simultaneous degossypolization and protein isolate preparation on bench scale to find out the extraction efficiency. The trials indicated that the extractability of the protein was in the range of 55-60 per cent (Table 1). There was no improvement in the extractability of protein when the peptization time was increased from 30 to 60 min. However, protein extractability could be increased to 70 per cent if the residue from the first extraction was

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TABLE 1. BENCH SCALE STUDIES ON THE PREPARATION OF COTTONSEED PROTEIN ISOLATE

Method	Extraction			Protein isolate			
	Protein		Extract-ability (100 × b) / a	Protein (%)	Gossypol		Available lysine (g/16gN)
	2 kg material (g) a	Peptized liquor (g) b			Free (%)	Total (%)	
Cottonseed flour							
30 min extraction	980	583	59.5	74.2	0.04	—	3.5
60 min extraction	980	554	56.5	73.0	0.04	—	—
Two stage extraction–30 min for each step[1]	980	716	73.3	76.3	0.05	—	3.4
Cottonseed flakes							
*Treatment—1**	980	518	52.8	87.7	0.05	0.5	3.3
Treatment—2**	—	—	—	—	0.03	0.08	2.6

1 The residue obtained after first extraction was re-peptized for further recovery of protein.

* Washed to remove colour, followed by wet grinding and extraction for 30 min.

** The extracted protein liquor from treatment 1 was subjected to bleaching by hydrogen peroxide.

repeptized for protein recovery. The protein obtained had very low free gossypol content (Table 1) but colour of the wet protein was dark green.

The method of washing and wet grinding followed by extraction gave rise to light coloured wet protein. The major drawback was the loss of solids (25 per cent) during the initial washing step. As a result of this loss, extractability of the protein was reduced to 52-53 per cent (calculated on the basis of meal taken). The free (0.03-0.05 per cent) and total gossypol (0.34-0.66 per cent) contents of protein were within the prescribed limits. The spray dried protein was dark brown. Hence, hydrogen peroxide treatment was employed to improve the colour. The bleached spray dried protein isolate was light yellowish.

The virtue of cottonseed as a source of protein is in its high lysine content (4.28/16 g N). However, lysine is easily rendered unavailable as it reacts with gossypol during processing i.e., expelling of oil[10]. Since there is no heat treatment during solvent extraction, lysine availability may not be affected in solvent extracted meal. In the present study, available lysine content of the protein isolate was found to be in the range of 3.3-3.5 g/16 g N; however, on peroxide treatment, it was reduced to 2.6 g/16 g N. The reduction in available lysine content due to peroxide treatment was also observed in case of groundnut protein[11]. There is not much information on the effect of hydrogen peroxide on ϵ-NH_2 groups of lysine. However, it was reported that during processing, protein undergoes structural changes and ϵ-NH_2 groups interact with other groups of protein forming cross-links and such reactions impair protein quality[12]. Possibly, peroxide treatment induced cross-linking lowering the availability of lysine.

TABLE 2. PROTEIN EFFICIENCY RATIO OF COTTONSEED PROTEIN ISOLATE (CPI)

Diet	Av. initial wt. (g)	Av. protein intake (g)	Av. gain in wt. (g)	PER
Experiment 1				
CPI	35.3	24.57	48.3	1.96±0.07*
H_2O_2 treated CPI	34.9	10.74	4.0	a
Skim milk powder	35.1	28.46	80.9	2.84±0.07*
Experiment 2				
CPI	42.4	27.64	60.2	2.17±0.08*
H_2O_2 treated CPI + methionine (0.22g)	42.4	10.80	-ve	b
H_2O_2 treated CPI + lysine (0.22g) + methionine (0.22g)	42.4	21.23	46.0	2.19±0.08**
Skim milk powder	42.4	27.85	73.2	2.62±0.08**

(Level of protein in diet = 10%; 10 male rats per group; assay period = 4 weeks)

a, b Failed to support growth. Hence, PER was not calculated.

*9 df; **27 df.

The protein isolate obtained from washed material was subjected to nutritional evaluation. The PER of the isolate was in the range of 1.96-2.2 while peroxide treated protein did not support growth at all (Table 2). Normally, methionine is rendered unavailable during peroxide treatment of protein[13]. However, supplementation of the peroxide treated protein with methionine alone was not found to be beneficial. Since available lysine also decreased, it was found that supplementation with both methionine and lysine was necessary for the improvement of growth and to increase PER to 2.2. In conclusion, the present studies have shown that the protein isolate prepared from gland rich cottonseed flour has good growth promoting value. On attempting to improve colour by hydrogen peroxide treatment, the nutritive value gets impaired.

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ETHANOL PRODUCTION FROM MIXED FRUIT JUICE OF DAMAGED GUAVA AND BANANA

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***Candida krusei* isolated from damaged guava and banana was compared with *Saccharomyces cerevisiae* CDRI and *S. cerevisiae* NCIM 3095 at different pH, nitrogen and phosphate levels for ethanol production from mixed fruit juice. *C. krusei* was found to have the same ethanol yield as *S. cerevisiae* CDRI NTG but required 20 hr more for fermentation of mixed juice as compared to CDRI NTG strain. Maximum biomass production in *C. krusei* was 2.1 g/l in 50 hr and 2.8 g/l in *S. cerevisiae* in 35 hr.**

A large quantity of guava and banana fruits are damaged every year due to disease and poor transport facilities. These damaged fruits can be effectively used for ethanol fermentation. Various workers used damaged fruit juices either unsupplemented[1,2] or supplemented with 0.1 per cent yeast extract[3] ammonium sulphate[4]. In this study, culture conditions were optimized for ethanol production using mixed fruit juices of damaged guava and banana.

The strain used for fermentation was isolated from the damaged banana at this laboratory and identified as *Candida krusei* by Dr. L. Rodrigues de Miranda, Yeast Culture Collection, Delft, Holland. The culture was maintained on MGYP agar slants at 4°C. The inoculum was prepared in MGYP medium. Fruit juices were prepared by cutting and blending the fruits separately and extracted by hand press. Nitrogen supplementation was done using $(NH_4)_2SO_4$ and phosphate supplementation by KH_2PO_4. These were added prior to sterilization. Fermentation was conducted in 11 conical flasks with a working volume of 100 ml at 30°C. The fermentation was terminated after 72 hr and the sugar[5], ethanol[6], nitrogen[7] and phosphate[8] were estimated after clarifying the fermented juice by centrifugation at 5000 r.p.m. for 15 min. Fermentation efficiency was calculated by the method of Bhandari[9]. The initial sugar, phosphate and nitrogen contents of damaged banana were observed to be 10.6, 0.024 and 0.168 per cent of wet weight respectively while the corresponding values for damaged guava were found to be 0.9, 0.009 and 0.016 per cent, respectively.

Fermentation efficiency and ethanol yield were maximum at pH 4.7 for the strain *C. krusei* and pH 5.0 for *S. cerevisiae* CDRI and NCIM 3095 (Fig 1).

A sugar to nitrogen ratio of 12.4:0.14 was found to be optimum for ethanol production by *C. krusei* and

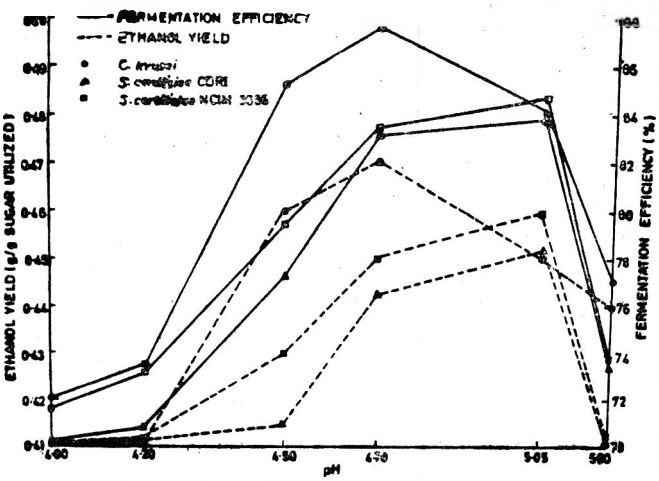

Fig. 1 Effect of pH on ethanol yield and fermentation efficiency of yeast strains

S. cerevisiae CDRI and 12.4:0.1 by *S. cerevisiae* NCIM 3095. Karni *et al*[10] have shown a comparable sugar: nitrogen ratio of 16:0.1 most suitable for fermenting juices.

Phosphate supplementation of mixed juices of damaged guava and banana registered an increase in fermentation efficiency in case of *C. krusei* and *S. cerevisiae* CDRI having optimum levels 0.75 and 1.0 g/l, respectively, an increase in fermentation efficiency due to activation of phosphofructokinase[11].

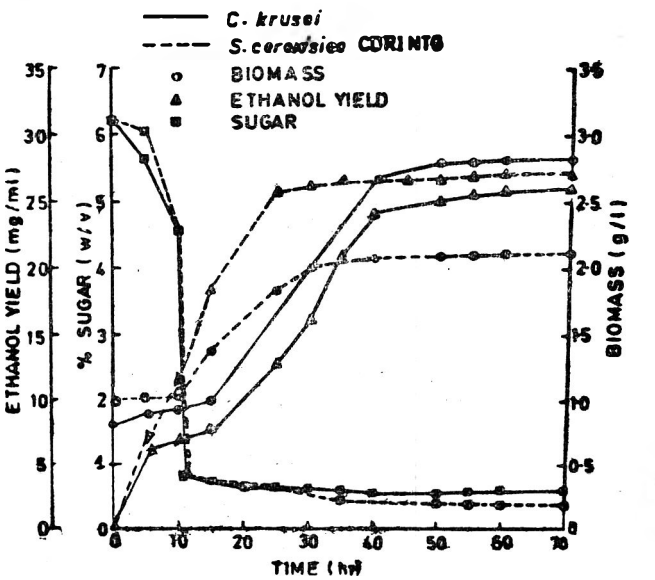

Fig. 2 Ethanol yield, biomass and sugar at different intervals of time

Maximum amount of biomass was obtained after 50 hr in *C. krusei* (2.8 g/l) and after 35 hr in *S. cerevisiae* CDRI NTG (2.1 g/l). Ethanol production was increased upto 55 hr in *C. krusei* and upto 35 hr in *S. cerevisiae* CDRI NTG (Fig. 2).

Thus, an attempt was made for utilizing mixed fruit juices of damaged guava and banana for ethanol fermentation.

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EVALUATION OF NEW GRAPE CULTIVARS FOR PROCESSING

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Two of the grape cultivars released from the Indian Institute of Horticultureal Research, Hessaraghatta, Bangalore were evaluated for raisin and juice extraction. 'Arkavati' being white seedless was found to be good for making raisin, while 'Arka Shyam' being black was adjudged good for juice extraction. Raisins from 'Arkavati' and juice from 'Arka Shyam' were found to be better than raisins of 'Thompson Seedless' and 'Concord' juice, respectively.

Work on grape improvement through breeding conducted for about 12 years at the Indian Institute of Horticultural Research, Hessaraghatta, Bangalore, resulted in releasing of four new hybrids[1], Viz. 'Arkavati', 'Arka Shyam', 'Arka Kanchan' and 'Arka Hans'. 'Arka Hans' and 'Arka Kanchan' both being seeded white grape varieties were not considered suitable for either raisin making or juice extraction. 'Arkavati' being white seedless var. was considered worth testing for its suitability for raisin making, while 'Arka Shyam' due to its shining bluish black colour was considered good for its evaluation for juice extraction. Currently, in India, 'Thompson Seedless' is the only cultivar recommended for making raisins, while juice is prepared from the cultivar 'Bangalore Blue' which is a coloured grape var. but has got high inherent acidity; therefore, they were used for comparison. Moreover, 'Concord' cultivar used in the U.S.A. and Europe for juice making was also used for comparison while evaluating 'Arka Shyam'.

About 5 kg grapes of each cultivar were harvested at commercial harvesting maturity stage during main season (i. e. February-March) from the experimental vineyard of the Indian Institute of Horticultural Research, Hessaraghatta, Bangalore. Bunches of 'Arkavati' and 'Thompson Seedless' cultivars after careful sorting (to remove diseased and damaged berries) were sulphured as per the method described by Amba Dan *et al.*[2] and were then put for natural drying under shade in a sell ventilated place. After about 4 weeks, when drying was over, dried berries i. e. raisins were separated manually from the rachis. Raisins were then given a quick wash with luke warm water to remove dirt, dust and pesticide residues from the surface of dried berries. This was followed by prompt drying under a current of air to remove moisture adhering to the surface. Raisins were then packed in 400 gauge polyethylene bags and were kept for about 2 weeks for moisture equalization. Finally, these raisin samples were evaluated for both physico-chemical constituents and sensory attributes like colour, texture and flavour.

For grape juice preparation, bunches were sorted, washed thoroughly to remove dirt, dust and pesticide residues and then drained completely. They were crushed in a grape crusher and heated to 70°C and kept at this temperature for half an hour to facilitate better colour extraction from the skins. Juice was extracted from the heated mass using basket press having drill cloth bag. Juice thus obtained was heated to 85°C, filled in clean dry bottles crown corked and heat pasteurized for 15 min in boiling water followed by cooling in air at room temperature. The bottles were kept at 5±1°C for argol precipitation. Finally, clear juice samples were syphoned out, heated to 85°C, rebottled and heat pasteurized as before. Juice samples were evaluated for physico-chemical and sensory attributes like colour, consistency and flavour.

Bunch weight and berry weight were recorded by a sensitive balance, while skin thickness of berries was measured with a screw gauge. An Erma hand refractometer (0-32 per cent) was used for determining total soluble solids (TSS). Necessary temperature corrections were made for these values. Elico pH meter and Ostwald viscometer were used for pH and viscosity determinations respectively. AOAC[3] methods were followed for determination of moisture, sugar, acidity, anthocyanins, tannins, sulphur dioxide and non-enzymatic browning (NEB). 'Amil' spectrophotometer was used for O.D. determination. For sensory analysis, hedonic scale was used keeping 30, 30 and 40 marks for colour,

TABLE 1. PHYSICO-CHEMICAL PARAMETERS OF GRAPE CULTIVARS 'ARKAVATI' AND 'THOMPSON SEEDLESS'

Parameter	Arkavati	Thompson Seedless
Bunch wt (g)	498.33±7.63*	740.00±13.23*
Berry wt (g)	1.80±0.10	2.50±0.10
Berry skin thickness (μ)	10± 1	25± 1
Total soluble solids (%)	23.13±0.81	20.00±1.00
Acidity (as tartaric %)	0.64±0.03	0.72±0.02

Berry flesh was firm in both the varieties.
*Mean ± SD

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consistency/texture and flavour respectively. Three replications were used both for analytical and sensory analysis. At each judging session, ten judges were invited.

Comparative values for various parameters in fresh grapes of 'Arkavati' and 'Thompson Seedless' cultivars are presented in Table 1. It is evident that in 'Arkavati', bunch size was smaller and less compact than that of 'Thompson Seedless' var. Berry size in terms of weight was less in 'Arkavati' and also its skin thickness was found to be only about half that of 'Thompson Seedless'. Further, higher total soluble solids and lower acidity values were observed in 'Arkavati' than in 'Thompson Seedless'. Based on these results, it is evident that 'Arkavati' gives better raisins than 'Thompson Seedless' variety.

Table 2. CHEMICAL COMPOSITION AND SOME PHYSICAL PROPERTIES OF RAISINS FROM 'ARKAVATI' AND 'THOMPSON SEEDLESS' GRAPE CULTIVARS

Constituents	Arkavati	Thompson Seedless
Moisture (%)	18.20±0.72*	20.20±0.72*
Sugars, reducing (%)	64.47±0.76	54.73±0.64
Total sugars (%)	68.60±0.53	59.77±0.68
Acidity (as tartaric %)	1.25±0.05	1.65±0.05
NEB (OD at 420 nm)	0.18±0.01	0.21±0.01
SO_2 (ppm)	190±6	228±2
Reconstitution ratio	2.18±0.07	1.52±0.08
Colour score (out of 30)	25±1	20±1
Texture score (out of 30)	26±1	22±2
Flavour score (out of 40)	31±1	25±1
Total sensory score	82±3	67±1.73

*Mean ± SD

Results of raisin analysis are presented in Table 2. Raisins from 'Arkavati' had higher sugar content, lower acidity, lesser NEB and greater reconstitution ratio than raisins from 'Thompson Seedless'. Because of these characteristics, 'Arkavati' raisins were considered superior in quality to those from 'Thompson Seedless'. This was supported by the results of sensory analysis; because of more bright golden yellow colour, soft texture and superior flavour, 'Arkavati' raisins were adjudged better than 'Thompson Seedless' raisins.

Total soluble solids, sugars, and anthocyanin contents were higher while acid content was less in 'Arka Shyam' (Table 3). In 'Concord', tannins and NEB recorded lowest values. It is thus evident that quality of juice from 'Arka Shyam' was the best followed by 'Concord' juice and 'Bangalore Blue' grape juice was the last. It was also observed that 'Arka Shyam' juice

Table 3. YIELD AND CHEMICAL COMPOSITION OF JUICES FROM 'BANGALORE BLUE, ARKA SHYAM AND CONCORD VARIETIES

Parameters	'Bangalore Blue'	'Arka Shyam'	'Concord'
Juice yield (%)	53.83±0.55*	67.20±0.26*	63.33±0.21*
T. S. S. (%)	20.00±0.20	24.00±0.20	21.20±1.06
pH	3.30±0.05	3.75±0.05	3.30±0.05
Sugars (total) (%)	16.40±0.40	18.60±0.20	17.63±0.15
Acidity (as tartaric %)	1.34±0.05	0.56±0.04	0.93±0.03
Viscosity (sec.)	87±2.64	58±2.00	68±3.46
NEB (OD at 420 nm)	1.25±0.05	1.50±0.10	1.00±0.00
Anthocyanins			
(OD at 525 nm)	0.30±0.05	0.32±0.02	0.34±0.02
(OD at 425 nm)	0.22±0.02	0.25±0.01	0.23±0.01
Tannins (OD at 620 nm)	0.62±0.02	0.57±0.06	0.31±0.01
Colour score (out of 30)	21±1	28±2	23±1
Consistency score (out of 30)	24±2	25±1	22±2
Flavour score (out of 40)	25±1.00	35±2.64	28±2.00
Total sensory score	70±2.64	88±2.64	73±2.64

* Mean ± SD NEB: Non enzymic browning.

scored highest for colour, consistency and flavour. Though colour and flavour of 'Concord' juice was considered better, its consistency was found to be thinner than that of 'Bangalore Blue'. From these findings, it is evident that the juice from cultivar 'Arka Shyam' is better than both 'Bangalore Blue' and 'Concord' juices.

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PHYSICO-CHEMICAL CHARACTERISTICS OF QUAIL-BROILER AND SPENT QUAIL MEAT

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Quality characteristics of dark and light meat from *Coturnix* quail aged 5 [quil-broiler] and 58 weeks [spent layer quail] were evaluated. Regardless of age, dark meat had higher moisture, ether extract, collagen contents, water-holding capacity, pH and shear force values than light meat while the reverse was true for crude protein, sarcoplasmic and myofibrillar proteins, Increase in age of the bird was accompanied by a decrease in moisture and organoleptic quality and an increase in crude protein, ether extract, collagen and shear force values in both the meat but myofibrillar proteins only in light meat. Cooking loss was relatively higher in spent layer carcass.

Within a decade of its introduction, the *Coturnix* quail has not only spread countrywide but also has been receiving increasing attention as a valuable source of meat and eggs. Assessment of quality factors of these products is essential to understand the relationship between closely related species, to get a better insight into the changes occurring during processing and/or storage and thus for their effective utilisation. However, no information on the influence of age on the quality characteristics of quail meat seems to be available in the literature. The results of a preliminary study on proximate composition, protein fractions and some functional properties both of dark and light meat from quail-broiler and spent layer quail are reported in this paper.

Dark (leg) and light (breast) meat from ten conventionally dressed carcasses belonging to each of the two age-groups, viz. 5-week old female quail-broiler and 58-week old spent layer quails, reared in cages under standard management conditions were manually removed, freed from visible fat, tendon and connective tissue. Respective meats were pooled and samples were taken in quadruplicate for analysis within 6 hr of exsanguination. Proximate composition[1], pH of ground meat by a Beckman pH meter, sarcoplasmic and myofibrillar proteins[2], collagen[3], non-protein nitrogen[4], extractable proteins (total of sarcoplasmic and myofibrillar proteins, expressed as per cent of total protein), water-holding capacity[5], shear force (force required to shear half-an-inch diameter cores of cooked meat using Barner-Bratzler shearpress) and cooking loss in 5 eviscerated carcasses cooked at 15 lb/in^2 pressure for 10 min were determined. A seven member sensory panel evaluated the meat for appearance, tenderness, flavour and overall acceptability on a 7-point Hedonic scale. The data were statistically analysed[6].

The effect of age on some physico-chemical attributes of quail meat are given in Table 1. Dark meat had significantly ($P<0.01$) higher moisture and lower crude protein contents than light meat in both the age groups. However, the differences in ether extract and total ash contents between meats were not significant. Increasing age of bird was associated with a decrease in moisture and an increase in crude protein and ether extract contents in both types of meat, exhibiting age associated changes in these constituents similar to that of other species of meat animals[7], including poultry[8]. Light meat had significantly lower pH values than dark meat probably due to faster rate of post-mortem glycolysis[9] which might be partly responsible for its relatively lower water-holding capacity than dark meat.

Extractable proteins differed significantly in spent quail meat. With increasing age, collagen content in both dark and light meat increased significantly ($P<0.01$), sarcoplasmic and myofibrillar proteins did not change in dark meat appreciably but exhibited a non-significant increase in light meat. The light meat contained lower collagen but higher contents of sar-

TABLE 1. PHYSICO-CHEMICAL CHARACTERISTICS OF QUAIL MEAT

Characteristics	Quail-broiler (5-wk)		Spent quail (58-wk)	
	Dark	Light	Dark	Light
Moisture (%)	$77.56^{a} \pm 0.15$	$76.42^{b} \pm 0.08$	$76.06^{b} \pm 0.20$	$74.60^{c} \pm 0.22$
Crude protein (%)	$18.27^{a} \pm 0.20$	$19.64^{b} \pm 0.34$	$19.62^{b} \pm 0.15$	$20.79^{c} \pm 0.13$
Ether extract (%)	$1.76^{ab} \pm 0.07$	$1.59^{a} \pm 0.03$	$2.51^{c} \pm 0.19$	$2.38^{bc} \pm 0.18$
Total ash (%)	$0.92^{a} \pm 0.14$	$1.02^{a} \pm 0.09$	$1.18^{a} \pm 0.14$	$0.96^{a} \pm 0.12$
pH	$6.22^{a} \pm 0.008$	$5.84^{b} \pm 0.01$	$6.24^{a} \pm 0.01$	$5.88^{b} \pm 0.01$
Extractable proteins (%)	$70.93^{a} \pm 0.39$	$70.87^{a} \pm 0.30$	$66.00^{b} \pm 0.13$	$71.14^{a} \pm 0.17$
Sarcoplasmic proteins (%)	$4.23^{a} \pm 0.10$	$4.45^{ab} \pm 0.06$	$4.28^{a} \pm 0.04$	$4.67^{b} \pm 0.13$
Myofibrillar proteins (%)	$8.73^{a} \pm 0.03$	$9.47^{b} \pm 0.11$	$8.67^{a} \pm 0.12$	$10.12^{b} \pm 0.21$
Collagen (%)	$2.06^{a} \pm 0.08$	$1.43^{b} \pm 0.04$	$3.38^{c} \pm 0.07$	$2.42^{d} \pm 0.08$
Non-protein N (%)	$0.28^{a} \pm 0.04$	$0.30^{a} \pm 0.03$	$0.31^{a} \pm 0.03$	$0.32^{a} \pm 0.02$
WHC (ml/100g)	$24.82^{a} \pm 0.21$	$19.56^{b} \pm 0.29$	$22.38^{a} \pm 0.39$	$18.02^{b} \pm 0.25$
Shear force (lb)	$2.48^{a} \pm 0.10$	$1.78^{a} \pm 0.11$	$4.92^{b} \pm 0.11$	$3.82^{c} \pm 0.17$
Overall acceptability*	$6.8^{a} \pm 0.20$	$6.6^{a} \pm 0.24$	$3.8^{b} \pm 0.20$	$4.0^{b} \pm 0.32$
Cooking loss (%)	$28.79^{a} \pm 0.63$		$30.56^{a} \pm 0.52$	

*7 = like very much, 1 = Dislike very much.
Means bearing the same superscript in each row do not differ significantly ($P<0.01$).

coplasmic and myofibrillar proteins than the former tissue in both the age groups. However, variability between meats or age of the bird in respect of non-protein nitrogen fraction was negligible. These results are in partial conformity with those reported for chicken meat[10] and the variability, particularly with respect to myofibrillar proteins, may be because of the differences in pre-and post slaughter conditions, extraction procedures and the species of bird.

Differences in shear force values between meats showed a close relation with their collagen contents. Significant ($P<0.01$) increase in shear force values of both dark and light meat from older birds might be attributable to the increased stability of collagen to heat degradation[11], less susceptability to the action of collagenase[12] and greater degree of its cross-linking between polypeptide chains with advancement of age. Cooking losses in carcasses from older birds were relatively higher than those from quail-broiler probably due to more loss of separable carcass fat in the former on cooking. Spent quail meat was rated organoleptically from tough (dark meat) to moderately tough (light meat) but had relatively higher flavour scores while quail-broiler meat had significantly ($P<0.01$) higher overall acceptability (Table 1).

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INSECTICIDAL ACTIVITY OF *EMBELIA RIBES* BURM.

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Various extractives of a medicinal plant, *Embelia ribes* were assayed against eggs and larvae of housefly, (*Musca domestica*) and adults of red flour beetle (*Tribolium castaneum*) and lesser grain borer, (*Rhyzopertha dominica*) to determine the presence of insecticidal principles. Powdered berries and their ethyl acetate extractives in culture medium reduced the per cent pupation of housefly when eggs were seeded. Against 48 hr old larvae, ethyl acetate extract and a crystalline fraction from it at 4% level resulted in 90 and 100% larval mortality respectively. Similarly, ethyl acetate extractive exhibited 76 and 67.6% mortality in *T. castaneum* and *R. dominica* adults respectively at 0.1% level in wheat. The crystalline portion from the ethyl acetate extractive resembled embelin and exhibited toxicity comparable to that of embelin against the three test insects.

Continuous use of synthetic insecticides to control various agricultural, household and storage insect pests has resulted in the development of insect resistance to such chemicals. Hence, there is a need to review our future strategies for pest control. Use of natural pesticides for pest control offers a promising alternative as these are easily biodegradable and comparatively safer to non-target species. Berries of *Embelia ribes* Burm. (a known medicinal drug[1]) have been reported earlier to possess insecticidal activity against larvae of *Musca domestica* L.[2] Present investigation deals with the assaying of various extractives of the berries and their comparison with the activity shown by embelin isolated separately from the berries.

Berries of *E. ribes* were procured locally, powdered and extracted serially in petroleum ether, ethyl acetate, alcohol and water. All the extractives were concentrated in a rotary flash evaporator at 35-40°C.

In preliminary experiments using eggs of housefly, powdered berries and extractives were tested at 15 or 30 per cent levels, while in experiments with 48 hr old larvae, extractives and embelin were assayed at 1, 2 and 4 per cent levels. Experimental procedures followed were the same as described earlier by the authors[3]. Toxicity of various extractives and embelin was evaluated on the basis of per cent pupation in the treatments as compared with the controls.

All the extractives and natural embelin isolated separately from the berries[4] were also assayed at 0.025, 0.05 and 0.1 per cent concentrations in wheat against *Tribolium castaneum* (Herbst.) and *Rhyzopertha dominica* (F.). Adult insects numbering 30 were exposed to treated and untreated wheat and mortality recorded after 3, 7 and 14 days. Effectiveness of the extractive was determined on the basis of per cent mortality observed after an exposure period of 14 days. All the experiments were conducted at 28±1°C and 65-75 per cent relative humidity.

Preliminary studies with housefly eggs indicated that pupation in culture medium incorporating powdered berries (15 per cent level) was 9.30 per cent and in ethyl acetate extractive (30 per cent level) was 5.30 per cent. Day of pupation was prolonged and pupae were smaller and weighed less than controls. Assay of different extractives against 48-hr old larvae revealed

TABLE 1. EFFICACY OF VARIOUS EXTRACTIVES OF EMBELIA RIBES AGAINST TRIBOLIUM CASTANEUM AND *RHYZOPERTHA DOMINICA* ADULTS

Extractives	% concn	% mortality after 14 days exposure	
		T. castaneum	*R. dominica*
Petroleum ether	0.025	0.00	6.33
	0.050	2.67	11.40
	0.100	3.95	14.80
Alcohol	0.025	5.33	6.33
	0.050	1.32	14.29
	0.100	5.40	7.50
Ethyl acetate	0.025	9.33	12.99
	0.050	30.70	64.50
	0.100	76.00	67.60
Black oily portion from ethyl acetate extractive	0.025	5.20	6.10
	0.050	5.26	10.80
	0.100	10.50	2.70
Crystalline portion from ethyl acetate extractive	0.025	93.59	79.22
	0.050	96.00	69.14
	0.100	96.00	78.30
Embelin	0.025	94.67	85.50
	0.050	94.67	70.00
	0.100	98.65	96.15
Control	—	2.53	8.10

All values are means from three replicates.

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that only ethyl acetate extractive and a crystalline fraction from it exhibited 90 and 100 per cent larval mortality at 4 per cent level. These results were comparable to the larval mortality (70 per cent) shown by embelin at 1 per cent level.

Ethyl acetate extractive at 0.1 per cent concentration exhibited 76 and 67 per cent mortality against adults of *T. castaneum* and *R. dominica* (Table 1) respectively. The crystalline fraction from ethyl acetate extractive gave high mortality of both the test insects and results were comparable with the efficacy shown by embelin even at 0.025 per cent concentration.

From the results obtained with various extractives, it was observed that only ethyl acetate extractive exhibited good activity against the three insects indicating the presence of active insecticidal principles. Further, when the ethyl acetate extract was fractionated, only the crystalline portion exhibited good activity which was comparable to that of embelin isolated separately. The crystalline fraction after purification resembled embelin in physical characteristics such as general appearance and m.p. Efficacy of natural embelin against *T. castaneum* and other stored product insect pests has been discussed earlier by the authors[5]. It can be concluded that possibly embelin is the only active insecticidal principle present in the berries.

Authors thank Dr. B. L. Amla, Director, of the Institute, and Shri S. K. Majumder, Deputy Director, for providing necessary facilities for the work.

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BOOK REVIEWS

Developments in Food Preservation: Edited by Stuart Thorne, Elsevier Applied Science Publishers, London and New York; 1985, pp: 315; Price: £ 36-00.

The present volume, third in the series, essentially concentrates on some of the major improvements in the fundamental understanding of the food preservation processes using thermal energy, save for a chapter on plant sanitation and another on application of fluidisation.

Chapter 1 discusses the current state of knowledge of the thermal distruction of spores, weaknesses which exist in the assumption that spore destruction is logarithmic, a number of methods available for calculation of the sterilization value or the processing time of the thermal process, and an insight into areas in which future research might be most useful.

Two Chapters (2 and 3) deal with vital aspects of food hygiene and microbiology, both essential to the effective and efficient prosecution of food preservation. Cleaning of food processing plant is not only essential to the safe operation of processes, but also contributes to the efficient operation of the plant. Extremely complex microflora of packaged meat must be controlled to provide a safe and acceptable product. Packaging and storage temperature limit the capability of development of vast array of species of the microorganism present on meat at the time of packaging. The influence of permeability of the packaging material, storage temperature and the curing ingredients on microbial growth, and conditions under which pathogenic organism can outgrow are critically examined.

Plant sanitation is vital for effective and efficient preservation of food. Sugar, mineral salts, fats and protein change on heating. Fouling of surfaces occur due to chemi-sorption and/or physical adsorption. Fouling of surfaces of food processing plants ranges from surface contamination to severe fouling of heat transfer surfaces. Chapter 3 summarises the basic mechanism of fouling methods of evaluation of surface cleanliness and method for plant cleaning including cleaning-in-place systems.

Chapter 4 is on the application of dielectric techniques as an alternative method for transferring heat to foods which is often more efficient than conventional heating by conduction, convection and/or radiation. The chapter covers the importance of the aqueous medium in the processing of foods, a survey of the work carried out to determine the electro-physical properties of raw materials in the food industry. Changes induced by the use of high- and ultra-high frequency, operations where dielectric energy could be applied and its advantages against conventional energy.

Chapters 5 and 6 are concerned directly with the thermal performance of heat exchangers. Falling film evaporators are used widely in concentration owing to short residence time, high heat transfer at low boiling point and minimum loss of the available temperature difference. Included in the Chapter 5 on falling-film evaporator, are the mathematical modelling of multiple effect falling-film evaporator, optimal design for preparing concentrated apple juice in multiple effect falling-film evaporator based on a design strategy of identical heat transfer surface in all the effects which was found to be more economical than that based on identical temperature difference and demonstration that when backward flow of feed is unsuitable, it is economical to pre-heat the feed in forward flow system.

The 6th Chapter deals with heat transfer and sterilization in continuous flow heat exchangers, principles of high or ultra high temperature and short time sterilization, kinetics of product quality changes and modelling of the sterilization effect for commercially available heat exchangers. A new concept of thermal-time distribution instead of the resident-time distribution as one characteristic of heat exchanger which cannot be verified experimentally, has been discussed.

The last chapter (Chapter 7) is a survey of the state-of-the-art of fluidisation in food processing which has found some application for several decades, while the high heat and mass transfer coefficients has found extensive application in the chemical industry, industrial developments and research in food processing. Processes, where fluidization await application in addition to gas-solid are the liquid-solid. The areas which have potential applications in food industry are roasting, freezing, freeze concentration, drying (including atmospheric freeze-drying), blanching, fermentation, extractions (liquid-solid or gas-liquid-solid), reverse osmosis and microbial or enzymatic reactions.

Printing, illustrations, get-up and binding of the book are good. It is a good source book of the present state of the art in the areas covered.

S. RANGANNA
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Alternative Sweeteners: Edited by Lyn O'Brien Nabors and Robert C. Gelardis Calorie Control Council, Atlanta, Georgia, Marce Dekker, Inc., New York. Basel, pp: X+355; 1985; Price: US $64.75 (US & Canada), $77.50 (All other countries).

The demand for non-caloric sweeteners has continuously been felt and several classes of materials have been explored over the years for their usefulness and safety. The literature in this field is vast and an authoriative and concise account of the various developments tn this area would be of considerable value to the novice as well as the expert. The multi-authored book of about 350 pages on "artificial sweeteners," edited by Nabors and Gelardi containing different aspects of the sweetener field brings the reader up-to-date in this area and is a valuable contribution. There are 16 different articles in this book covering a whole host of sweeteners of past and present interest. The special feature of this book is the availability in one place of various types of information such as current manufacturing practices, physico-chemical characteistics, stability, utility in food and beverage systems, safety aspects (metabolic studies, pharmacology, toxicology, carcinogenecity, mutagenecity, teratology), regulating and marketing aspects of important sweeteners like sachharin, cyclamate and aspartame. Recent developments in the use of Thaumatin as a sweetner have also been covered. There is very useful information with respect to dihydro-chalcone sweeteners, L-sugars, polyalcohols, crystalline fructose, steriosides, etc. Each one of these sweeteners is dealt with by an expert in this area and the vast literature has been admirably condensed.

I storongly recommend this book to all scientists who would be interested in getting a quick and authoritative glimpse of the artificial sweetener field.

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1. *Environmental Health Criteria* 66: Kelevan; World Health Organisation (WHO), Geneva, 1986; pp: 32; Price: Sw. fr. 6.
2. *Environmental Health Criteria* 67: *Tetradifon:* World Health Organisation (WHO), Geneva, 1986; pp: 47; Price. Sw. fr. 7.

The International Programme on Chemical Safety (IPCS) is a joint venture published by the United Nations Environment Programme, the International Labour Organization and the World Health Organization in Geneva. The main objective of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment. Supporting activities include the development of epidemiological, experimental laboratory, and risk-assessment methods that could produce internationally comparable results and the development of manpower in the field of toxicology. To date, over sixty six titles on various chemicals have been published containing the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the United Nations Environment Programme.

The subject matter includes identity, physical and chemical properties of the compounds, analytical methods, sources of human and environmental exposure; Environmental transport distribution and transformation, abiotic degradation; Environmental levels in water, soil, food and animal feeds; Kinetics and metabolism; distribution, storage, metabolic transformation and excretion; effects on organisms particularly aquatic, terrestrial organisms; effects on experimental animals and *in vitro* test systems; oral, dermal, inhalation exposures, studies on reproduction, mutagenecity, carcinogenecity and evaluation of health risks for man and effects on the environment.

Kelevan is an organochlorine, a chlordecone derivative which is used in some countries for the control of *Leptinotarsa decemlineata* on potatoes and the banana root borer *Tanymecus palliatus*. As the available data on Kelevan is sparse it is difficult to arrive at an informed evaluation of its impact on the environment. As the data ont oxicology of chlordecone is somewhat similar to kelevan, this data should be useful unless further data to the contrary become available. This would mean that unless kelevan is indispensable, it is not recommended.

With the acaricide tetradifon, more information is needed on the metabolism, on the effects on reproduction and on long-term toxic effects including carcinogenecity. It is advised that the purity of the products registered and used be ascertained since the contamination by some chlorinated compounds may increase its toxicity.

The publications are very valuable to research workers in pesticide toxicology and regulatory bodies.

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Modern Control Techniques for the Processing Industries, Chemical Industries Series, Vol. 23, by T. H. Tsai, J. W. Lane and C. S. Lin, Marcel Dekker, Inc., 270 Maiden Avenue, New York, 10016; 1986; pp. 296; Price: US $59.75 (US & Canada), $71,50 (All other countries).

'Modern Control Techniques for Processing Industries' is a reference book on Computer-based Control Systems which deals with process control fundamentals and applications. The Introductory Chapters on 'Digital Control Systems', 'Programmable Logic Controls' and 'On-line Continuous Measurement System' give greater in-depth study and process control techniques and will provide enough source material for chemical, process and electronic engineers and Managers in chemical, pharmaceuticals and other processing industries. However, the Chapter on 'Process Control Computer' is to elementary and enough literature is available at present. The Chapter on 'Process Model and Control Concept on Algorithm Design' is a good digest on modelling techniques and model construction and illustrates a practical method for constructing meaningful models to facilitate control strategy, testing and evaluation of process plant operations. The topic on 'Supervisory Control System' covers all major aspects of supervisory control systems including implementation of multilevel control strategy with a Honeywell computer using a process stimulator for complicated processes. The book is a good reference work for practising engineers in the field of chemical engineering and instrumentation.

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AFST(I) News

Hyderabad Chapter

The Annual General Body Meeting was held on 27th February 1987. The following office bearers were unanimously elected for 1987.

President:	Dr. M. M. Paulose
Vice-President:	Mr. Surendra Kumar
Hony. Secretary:	Dr. Y. S. R. Sastry
Jt. Secretary:	Mr. H. K. Guru Raja Rao
Hony. Treasurer:	Dr. R. B. N. Prasad

Calcutta Chapter

The 26th Annual General Body Meeting of the Chapter was held on 16th January 1987. The following offiice bearers were unanimously elected for 1987–88.

President:	Mr. S. N. Mitra
Vice-Presidents:	1. Mr. R. N. Ghosh
	2. Mr. Om. P. Dhamija,
Hony. Secretary:	Mr. Bhudeb Gupta
Jt. Secretaries:	1. Dr. A. K. Guha
	2. Smt. Sikha Mukhopadhyay
Hony. Treasurer:	Mr. P. L. Agarwal
Asst Treasurer:	Mr. Rajesh Mehta

Madras Chapter

The Annual General Body Meeting was held on 9th April 1987. The office bearers for 1987 are as follows:

President:	Dr. T. S. Santhanakrishnan
Vice-President:	Dr. P. G. Adsule
Secretary:	Shri Hukum Singh Kaintura
Jt. Secretary:	Shri N. Ibrahim
Treasurer:	Shri K. L. Sarode
Editor:	Mrs. Malathi Mohan

ASSOCIATION OF FOOD SCIENTISTS & TECHNOLOGISTS (INDIA)

CFTRI Campus, Mysore-570 013, India

NOMINATIONS FOR AFST(I) AWARDS FOR 1987

Nominations for the following awards of the AFST(I) for the year 1987 are invited. All nominations should be sent by Registered post, so as to reach Dr T. S. S. Rao, Honorary Executive Secretary, Association of Food Scientists and Technologists (India), CFTRI Campus, Mysore-570 013, before 31 October 1987. The awards will be presented during IFCON–88 at Mysore on February 18, 1988.

PROF. V. SUBRAHMANYAN INDUSTRIAL ACHIEVEMENT AWARD

The guidelines for the award are:

(i) Only Indian nationals with outstanding achievement in the field of Food Science and Technology will be considered for the award.

(ii) The nominee should have contributed significantly to the enrichment of Food Science and Technology, and the development of agro-based food and allied industries in India.

(iii) The nomination duly proposed by a member of the Association must be accompanied by the biodata of the candidate highlighting the work done by him for which he is to be considered for the award.

(iv) The awardee will be selected by an expert panel constituted by the Central Executive Committee of the Association.

The envelope containing the nominations along with biodata and contributions (five copies) should be superscribed "Nomination for Prof. V. Subrahmanyan Industrial Achievement Award—1987".

LALJEE GODHOO SMARAK NIDHI AWARD

The guidelines for the award are:

(i) The R & D group/person eligible for the award should have contributed significantly in the area of Food Science and Technology in recent years with a good standing in his/her field of specialisation.

(ii) The nominee(s) should be duly sponsored by the Head of the respective Scientific Institution and the application for this award should highlight complete details of the contributions made by the candidate and their significance.

(iii) The awardee(s) will be selected by an expert panel constituted by the Central Executive Commitee of the Association.

The envelope containing the nominations (five copies) should be superscribed "Nomination for Laljee Godhoo Smarak Nidhi Award 1987".

SUMAN FOOD CONSULTANTS TRAVEL AWARD

This award is instituted in the name of M/s Sumen Food Consultants, New Delhi, to be awarded to a student pursuing Post Graduate Degree/Diploma courses in Food Science/Technology in any recognised University in India. The award will enable the selected student to attend IFCON–88 at Mysore during February 18-23, 1988.

The Award will be decided based on the best essay to be submitted by the applicant "MICROWAVE HEATING SYSTEM FOR FOOD PROCESSING." Five copies of the essay containing 15–20 pages (A4 size) of typed matter with appropriate bibliography and a certificate from the head of the department under whom the student is working should be enclosed along with the application. The envelope containing the above documents should be superscribed "Suman Food Consultants Travel Award–1987".

BEST STUDENT AWARD

The award is to be given to a student having a distinguished academic record and undergoing the final year course in Food Science and Technology in any recognised University in India. The aim of the award is to recognise the best talent in the field and to encourage excellence amongst the student community.

The guidelines for the Award are:

(i) The applicant must be an Indian national.

(ii) He/she must be a student of one of the following courses:

(a) M.Sc. (Food Science)/(Food Technology)

(b) B.Tech., B.Sc. (Tech), B.Sc. (Chem. Tech) with Food Technology specialisation.

(iii) He/she should not have completed 25 years of age on 31st December 1987.

Heads of the Department of Food Science and Technology in various Universities may sponsor the name of one student from each institution supported by the candidate's biodata, details starting from high school onwards, including date of birth and post-graduate performance to date (five copies).

The envelope containing the nomination should be superscribed "Nomination for Best Student Award–1987".

YOUNG SCIENTISTS AWARD

This award is aimed at stimulating distinguished scientific and technological research in the field of Food Science and Technology amongst young scientists in their early life.

The guidelines for the Award are:

1. The candidate should be an Indian national below the age of 35 years on 31st December 1987, working in the area of Food Science and Technology.

(i) The candidate should furnish evidence of either:

(a) Original scientific research of high quality, primarily by way of published research papers and (especially if the papers are under joint authorship) the candidate's own contribution to the work.

OR

(b) Technological contributions of a high order, as reflected by accomplishments in process design etc., substantiated with documentary evidence.

The application along with details of contributions of biodata (five copies) may be sent by registered post with the envelope superscribed: "Nomination for Young Scientist Award–1987".

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

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