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ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS

(INDIA)

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3. To promote the profession of Food Science and Technology.

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Each Synopsis gives the essential information regarding the method of experimentation, the results obtained and the conclusions reached, whereas tables, figures and detailed discussion of results (by the author) have been omitted.

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Proceedings of the Symposium on "Fats and Oils in Relation to Food Products and their Preparations", held at C.F.T.R.I. Mysore-13, 3–4th June 1976.

This two-day Symposium on the uses of fats and oils in food was organised jointly by the Association of Food Scientists and Technologists (India), the Oil Technologists' Association of India (SRB), and the Central Food Technological Research Institute Mysore. It belongs among the series of quasi-annual Symposia organised on all India basis at the CFTRI, since the last ten years or so. Most of the leading research workers in the field of oils and fats have participated in it. They come from leading National Laboratories, as well as from University Departments and Industries.

The Proceedings consist mainly of 42 papers presented in Six Sessions; viz.,

Session I—Raw Material Survey of Resources and Newer Sources of Fats & Oils .....(6 papers)

Session II—Processing, Hydrogenation, Emulsification, Inter-esterification, Refining and Modification of Fats and Oils. .....(7 papers)

Session III—Fat-based Food Products-Indian Confectionery, Deep-fat Fried Products, Margarine, Pickles, Salad Cream, Mayonnaise, Butter, Cheese and Ghee. .....(8 papers)

Session IV—Nutrition and Toxicity. .....(7 papers)

Session V—Autoxidation, Antioxidants and Storage Characteristics of Fats and Oils. .....(8 papers)

Session VI—Chemistry, Analytical Techniques, Adulteration and Quality Control .....(6 papers)

The topics covered in the special lectures are: "Mechanism of blood-cholesterol lowering effects of unsaturated fatty acids" and "Producing and marketing a low-cost, volume food".

Thus a very wide range of subjects have been covered. Though comprehensiveness cannot be expected from a Demy—quarto volume of 155 pages, the reader will find a lot of information that would help to modernize the fats and oils processing industry in India. Considered as a compact report on the state of oils and fats technology in India at the commencement of the last quarter of the twentieth century, this publication must be considered as indispensable for all Scientists and Technologists, as well as institutions that work on the field of edible fats and oils.

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JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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Research Notes

Whey Protein Profile of Buffalo Milk

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Manuscript Received 18 *July* 1978; *Revised* 12 *December* 1978

Chromatography of buffalo milk whey on Sephadex G-75 and DEAE-Sephadex A-50 indicated presence of three variants of beta-lactoglobulin having two of these variants, have similar electrophoretic mobilities as bovine beta-lactoglobulin A and C were observed in all samples studied, but the faster moving variant was not present in the milk of certain individual buffaloes. In the alpha-lactalbumin fraction of buffalo milk two major and two minor components were observed. These components of alpha-lactalbumin were found to behave similar to the 'total' alpha-lactalbumin of bovine milk in modifying the galactosyl transferase function of "A" protein of bovine milk to give lactose synthetase activity. Distribution of various components of whey proteins in buffalo milk was also studied by 'Discontinuous polyacrylamide gel plate electrophoresis' technique.

Whey proteins of buffalo milk has been reviewed earlier by Laxminayana and Dastur[1] and that of bovine milk by McKenzie[2]. Most of the investigators have studied various components of whey proteins by using salt fractionation and paper electrophoresis[3,4] Thus, early reports of Sen and Sinha[5], Bhattacharya *et al*[6]. and Mawal *et al*[7]. indicated that the buffalo milk beta-lactoglobulin and alpha-lactalbumin do not have genetic variants. In recent years, polyacrylamide gel electrophoresis is known to be an effective technique for good resolution of whey protein components[8-10], and has been used in this investigation to study the whey proteins of buffalo milk.

Materials and Methods

Samples of buffalo milk were obtained from Murrah buffaloes maintained at the National Dairy Research Institute, ICAR, Karnal. Chemicals and reagents used in this study were of the highest purity commercially available.

Whey samples: To 1 l. of milk sample at 20°C, 264g of ammonium sulphate was gradually added over a period of 40 min with constant stirring. The precipitate of casein and entrapped fat so formed was removed by filtering through Whatman No. 1 filter paper. The filtrate (whey) obtained was desalted on a Sephadex-G-25 column (5×51 cm) in batches of 150 ml. Desalted whey was then freeze dried and preserved at 4-6°C for further analysis.

Chromatography on Sephadex G-75: The method of Hopper and McKenzie[11] was used for chromatography of whey proteins on Sephadex G-75. About 0.8 g of freeze dried whey was dissolved in 10 ml of imidazole-hydrochloric acid buffer (pH 6.3, I=0.043) and chromatographed on a Sephadex G-75 column (2.6×84 cm). It was eluted with the same buffer at a flow rate of 30 ml/hr and 5 ml fractions were collected. The effluent was monitored for proteins by measuring optical desnity at 280 nm in a Beckman DU spectrophotometer.

Chromatography on DEAE-Sephadex A-50: This was also carried out according to the method of Hopper and McKenzie[11]. Fractions high in protein content obtained by the chromatography of whey on Sephadex G-75, were applied to a column of DEAE-Sephadex A-50 (2.6×20 cm), and eluted with the imidazole-hydrochloric acid buffer (pH 6.3, I=0.043) using a linear gradient of sodium chloride (0.0 to 0.10 M). Fractions of proteins so obtained were dialyzed against several changes of distilled water (4-6°C) till free of sodium chloride, and finally freeze dried.

Distribution of proteins in milk and whey: Gross distribution of casein and whey protein components in in milk and whey samples was done according to the method of Nagasawa *et al*[12]. Kjeldahl nitrogen was determined according to the method of McKenzie[2]. Distribution of whey protein components was studied by the Discontinuous Polyacrylamide Gel Plate Electrophoresis (DPGE) technique. The concentration of individual components of whey proteins was determined densitometrically from the area under each peak. Identification of various protein components resolved by DPGE was carried out using purified protein samples, as described earlier[10]. Starch gel electrophoresis (SGE) was according to the method of Hopper.[13]

Lactose-synthetase activity: The spectrophoto-metric method of Ebner *et al.*[13] was used for determination of the lactose synthetase modifier activity of alpha-lactalbumin fractions obtained by the chromatography of whey

samples on the Sephadex G-75 and DEAE-Sephadex A-50. The blank contained all the reagents used in the assay except the substrate. The assay estimated the formation of UDP by coupling with pyruvate kinase and lactate dehydrogenase and measuring the oxidation of NADH. Galactosyl transferase was isolated from buffalo milk by the method of Ebner *et al*[14]. The lactose synthetase modifier activity of buffalo alpha-lactalbumin fractions was expressed as percentage of the activity of bovine lactose synthetase system.

Results and Discussion

By chromatographing whey samples of buffalo milk on Sephadex G-75, whey proteins were resolved into four fractions. These fractions could be resolved further by chromatography on DEAE-Sephadex A-50, as discussed latter.

Chromatography of Buffalo whey on Sephadex G-75: The elution profile of total whey proteins from Sephadex G-75 column (2.6×84 cm) at pH 6.3 is given in Fig. 1. It may be observed that four fractions were obtained. The components of whey proteins present in these fractions, as revealed by DPGE, were as follows:

Fraction I: Proteins present in this fraction were eluted in the void volume of Sephadex G-75 column. From densitometric, patterns, these components were found to have electrophoretic mobilities similar to those of bovine serum albumin, immunoglobulins and lactoferrin, in the descending order of electrophoretic mobilities.

Fraction II: From scanning patterns, presence of three components was indicated in this fraction, one of these components displayed slightly faster electrophoretic mobility compared to the bovine beta-lactoglobulin A, while the other two components had similar electrophoretic mobilities as the A and C variants of bovine beta-lactoglobulins.

Fraction III: It was observed that the whey proteins present in this fraction displayed three bands on DPGE. The electrophoretic mobilities of two major bands were found to be comparable with the bovine alpha-lactalbumin A and B, while that of the minor components was slightly faster than bovine alpha-lactalbumin A.

Fraction IV: It was observed that this fraction consisted of two protein components. One of the these components exhibited slightly faster electrophoretic mobility than bovine alpha-lactalbumin A, while the other component had slightly slower mobility compared to the bovine alpha-lactalbumin B.

These fractions II, III, and IV of ammonium sulphate buffalo whey obtained by chromatography on Sephadex G-75 column, as described above, were further chromatographed on DEAE-Sephadex A-50 column (2.6×20 cm), using a linear gradient of sodium chloride:

Chromatography of Fraction II on DEAE-Sephadex A-50: The pattern of elution is indicated in Fig. 2. DPGE of the first fraction revealed presence of two protein components. One of these components displayed slightly faster electrophoretic mobility while the other had similar mobility compared to the bovine beta-lactoglobulin A.

The scanning patterns of the second fraction of DEAE-Sephadex A-50. chromatography indicated presence of two components. One of these components had similar electrophoretic mobility as bovine beta-lactoglobulin A, while the second component had an electrophoretic mobility comparable to beta-lactoglobulin C of bovine milk.

Chromatography of Frection III on DEAE-Sephadex A-50: Proteins present in Fraction III were eluted in three fractions by chromatography on DEAE-Sephadex A-50, as shown in Fig. 3. Electrophoresis of first peak by DPGE indicated presence of one protein fraction which had slightly faster electrophoretic mobility compared to bovine alpha-lactalbumin A.

The second peak indicated presence of two major components, which displayed same electrophoretic mobilities as bovine alpha-lactalbumins A and B.

The third peak indicated the presence of two major and one minor component. Two major components had similar electrophoretic mobilities as those of bovine

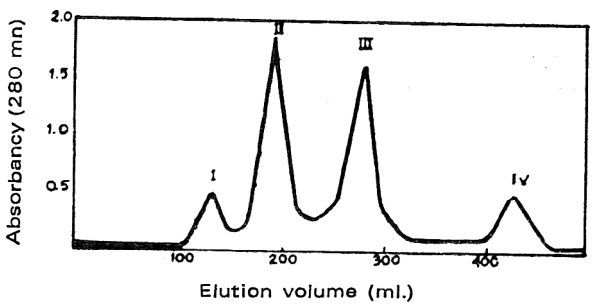

Fig. 1. Elution profile of buffalo whey proteins from chromatography on sephadex G-75 column (2.6×84 cm) at pH 6.3.

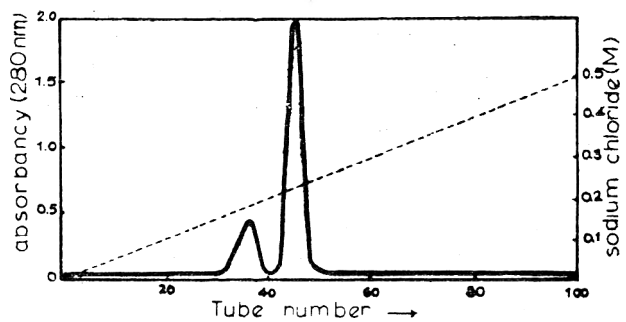

Fig. 2. Elution profile for the buffalo beta-lactoglobulin on a DEAE-Sephadex A-50 column of size 2.6×20 cm at pH 6.3 using a linear gradient of sodium chloride.

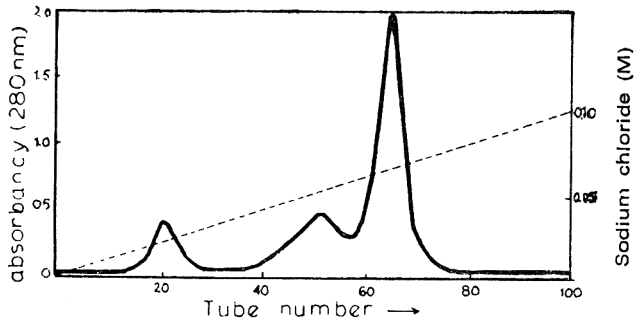

Fig. 3. Elution profile for the separation of buffalo alpha-lactalbumin (fraction III of whey from Sephadex G-75) on a DEAE Sephadex A-50 column (2.6×20 cm) at pH 7.4 using a linear gradient of sodium chloride.

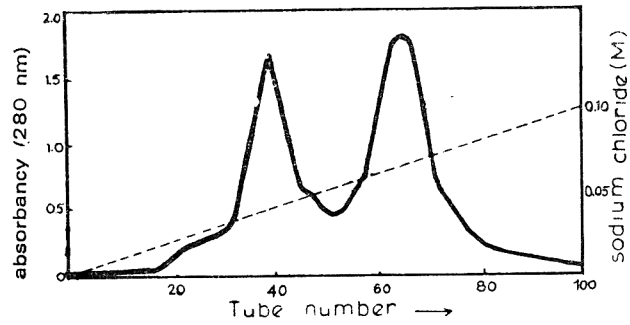

Fig. 5. Elution profile for the separation of buffalo alpha-lactalbumin (fraction IV of whey from Sephadex G-75) on a DEAE-Sephadex A-50 column (2×20 cm) at pH 7.4 using a linear gradient of sodium chloride.

alpha-lactalbumin A and B, while the minor components displayed slightly slower mobility than bovine alpha-lactalbumin A.

Results obtained by employing the DPGE technique in this case were compared with the SGE technique of Hopper[13]. As shown in Fig. 4, proteins present in the first peak were resolved into a single band. Proteins present in the second and third peaks were resolved into two bands. In this manner, resolution of alpha-lactalbumin components seem to depend upon the method of electrophoresis employed.

Chromatography of Fraction IV on DEAE-Sephadex A-50: Proteins present in Fraction IV were eluted in two peaks by chromatography on DEAE-Sephadex column, as may be seen from Fig. 5. DPGE of first peak revealed presence of two components. One of these components had faster electrophoretic mobility compared to bovine alpha-lactalbumin A, while the other had slightly slower electrophoretic mobility compared to bovine alpha-lactalbumin B. In the second peak also, presence of two components was indicated by DPGE having similar electrophoretic behaviour as those of first peak.

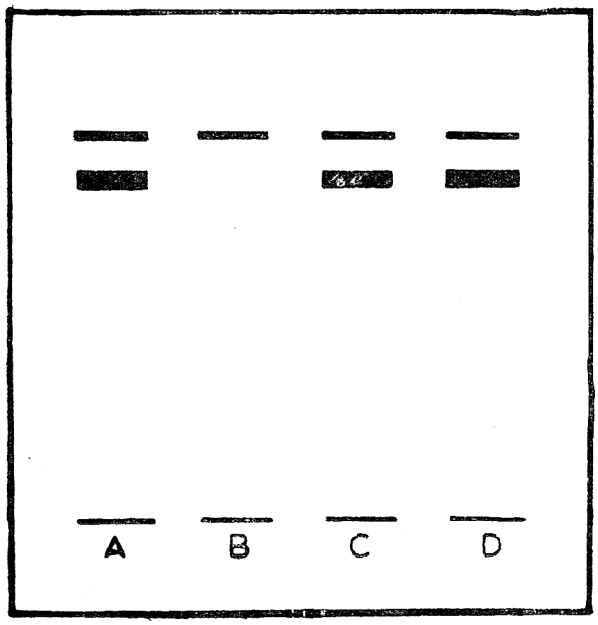

Fig. 4. Starch gel electrophoresis of buffalo alpha-lactalbumin electrophoresis carried out at 7 v/cm for 4 hours using sem discountinuous buffer system (ph 7.7). A: fraction III of ammonium sulphate whey from Sephadex G-75; B: fraction 1 of DEAE-Sephadex A.50; C: fraction 2 of DEAE-Sephadex A-50;D: fraction 3 of DEAE-Sephadex A.50.

Lactose synthestase modifier properties of the protein components present in Fractions III and IV: Since protein components present in Fraction III and IV (from the chromatography of buffalo whey on Sephadex G-75) resembled bovine alpha-lactalbumin in the chromatographic and electrophoretic behaviour, further work was undertaken to ascertain whether these components performed the biological function of transforming beta-4-galactosy transferase from N-acetylactosamine synthetase to 'lactose synthetase'. Various components obtained by the chromatography of Fraction III and IV on DEAE-Sephadex A-50, as described in the preceeding text, were assayed, and the results obtained are given in Table 1. It was observed that all these components behaved similar to the total bovine alpha-lactalbumin

TABLE 1. 'LACTOSE SYNTHETASE' ACTIVITY OF DIFFERENT FRACTIONS OF BUFFALO ALPHA-LACTALBUMIN

Fraction from Sephadex G-75	Fraction from DEAE-Sephadex	% lactose synthetase activity with bovine alpha lactalbumin as 100
III	1st	98.11
III	2nd	98.41
III	3rd	99.24
IV	1st	97.28
IV	2nd	97.67

TABLE 2. COMPONENTS OF BUFFALO BETA-LACTOGLOBULIN OBSERVED BY THE CHROMATOGRAPHY OR AMMONIUM SULPHATE WHEY ON SEPHADEX G-75 AND DEAE-SEPHADEX A-50

Component of buffalo beta-lacto-globulin	Electrophoretic mobility on DPGE compared tobovine lactoglobulin variants	Elution on Sephadex G-75 chro-matography	Elution on DEAE-Sephadex A-50 chromatography
Fast	faster than beta-lactoglobulin A	Fraction II	Ist fraction
A	same as beta-lactoglobulin A	Fraction II	Ist & 2nd fraction
B	same as beta-lactoglobulin C	Fraction II	2nd & 3rd fraction

in modifying the galactosyl transferase function of 'A' protein to give lactose synthetase activity (Table 2).

Heterogeneity of buffalo beta-lactoglobulin: The results obtained during the course of this investigation indicate that contrary to earlier published reports[5-7], buffalo beta-lactoglobulin is not homogeneous. Presence of three variants was indicated by polyacrylamide gel electrophoresis and ion exchange chromatography. These chromatographic studies on the heretogeneity of buffalo beta-lactoglobulin confirm our preliminary results obtained by directly resolving samples of buffalo milk employing DPGE technique[10]. Additional studies indicated that the 'fast variant' of buffalo beta-lactoglobulin was not present in the milk of 8 out of 48 milk samples studied from the individual buffaloes. It may be, therefore, speculated that this variant could be genetically controlled.

TABLE 3. COMPONENTS OF BUFFALO ALPHA-LACTALBUMIN OBSERVED BY THE CHROMATOGRAPHY OF AMMONIUM SULPHATE WHEY ON SEPHADEX G-75 AND DEAE-SEPHADEX A-50

Component of buffalo alpha-lactal-bumin	Electrophoretic mobility on DPGE compared to bovine alpha-lactalbumin variants	Elution on Sephadex G-75 chro-matography	Elution on DEAE-Sephadex A-50 chromatography
Minor (fast)	faster than alpha-lactalbumin A	Fraction III	1st fraction
		Fraction IV	1st & 2nd fraction
Major A	same as alpha-lactalbumin A	Fraction III	2nd & 3rd fraction
Major B	same as alpha-lactalbumin B	Fraction III	2nd & 3rd fraction
Minor (slow)	slower than alpha-lactal-bumin B	Fraction IV	1st & 2nd fraction

TABLE 4. PROTEIN DISTRIBUTION IN BUFFALO MILK

Particulars	Percentage
A. GROSS PROTEIN DISTRIBUTION[1]	
Caseins	3.26±0.14
whey proteins	0.81±0.04
total proteins	4.07±0.09
B. DETAILED DISTRIBUTION OF WHEY PROTEINS[2]	
beta-lactoglobulin (fast)	1.81±0.23
beta-lactoglobulin A	10.86±0.42
beta-lactoglobulin C	21.49±0.39
alpha-lactalbumin A	21.04±0.62
alpha-lactalbumin B	13.12±0.91
serum albumin	13.57±0.72
others	20.36±0.82

1. Protein content in milk as g. /100 ml.
2. Relative distribution of whey protein components.

Heterogeneity of buffalo alpha-lactalbumin: From the results obtained during the course of this study, it appears that two major and two minor components may be present in buffalo alpha-lactalbumin. The chromatographic behaviour of these components is given in Table 3. It was observed that all the fractions of buffalo alpha-lactalbumin obtained by chromatography on Sephadex G-75 and DEAE-Sephadex A-50 displayed lactose synthetase activity similar to the bovine alpha-lactalbumin (Table 1). In the earlier published studies using paper electrophoersis techniques for the resolution of whey proteins, presence of variants was not observed for this fraction of buffalo milk[5,6]. However, by employing DPGE technique for the resolution of whey proteins directly from milk or whey samples, presence of two variants having similar electrophoretic mobilities as bovine alpha-lactalbumin A and B was indicated[10].

Distribution of Whey Proteins in Buffalo milk: The application of electrophoretic technique for determining the distribution of protein components in milk was first reported by Larsen and Rolleri[9]. In this investigation, densitograms obtained by the DPGE were employed to study the whey protein distribution in buffalo milk. Average values from 30 samples of pooled milk are given in Table 4. Earlier, by employing salt fractionation technique, Ghosh and Anantakrishnan[3] reported range for albumin and globulin content as 0.34 to 0.36 per cent and 0.158 to 0.177 per cent respectively. Ganguli *et al*[3] reported mean values of proteose peptone, whey protein and non protein nitrogen contents of buffalo milk as 190, 670 and 155 mg/100 ml respectively.

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Changes in Proteins and Viscosity of Goat Condensed milk During Storage

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Condensed milk prepared on a laboratory scale from goat's milk was evaluated for changes in the major milk proteins and viscosity during storage for 4 months. Percent distribution of goat milk protein into casein, whey proteins, proteose-peptone and non-protein nitrogen fraction was 73.76, 19.74, 4.95 and 1.57, respectively. There was no change in the α-casein level (36.54%) on forewarming, but concentration of β-casein decreased from 63.54 to 58.86%. No appreciable difference in the viscosity of raw and forewarmed milk was observed. However, there was considerable increase (14.27 to 49.98 poises) in the viscosity of the condensed milk on storage. By electrophoresis it was observed that on storage two new protein fractions appeared. The relative proportion of α -and β-casein decreased on storage, whereas reverse was observed in case of new fractions. There was no change in the total protein content but casein showed a decrease in concentration during storage. On the other hand, proteose-peptone and non-protein nitrogen fractions increased with increase in storage period.

In some countries goat milk is being processed for the manufacture of specific milk products such as yoghurt, dried milk and evaporated milk[1,2]. In Turkey, Cyprus, Greece and France goat milk is generally used for cheese manufacture[1-4].

In India, goat milk has a great potential not only as a market milk but also for the preparation of products. So far no systematic attempts have been made in such a direction. The results on the preparation of condensed milk from goat milk on a laboratory scale are now reported. Physico-chemical data on the changes in such a product during storage are also presented.

Materials and Methods

Pooled milk samples of Beetle goats maintained at the Institute herd were used to prepare condensed milk. Hydrolysed starch of Smithes grade and Bromophenol blue were obtained from B.D.H. All the other chemicals were of analytical grade.

Preparation of condensed milk: Five samples of condensed milk were prepared from goat milk on laboratory scale for the study of their properties and effect of storage on the change occurring in the condensed milk. Fresh goat milk was forewarmed to 115°C by placing 250 ml in an Erlenmeyer flask in an autoclave and heating for 11 to 12 minutes. The milk was cooled to room temperature in 5 to 6 min.

Condensation of forewarmed milk: The forewarmed milk was condensed in a rotary vacuum eavporator at an absolute pressure of 0.3 u of Hg.

The solids content of the initial milk and condensed milk was determined by Bausch and Lomb refracto-

meter. When the total solids of the condensed milk reached near 31 percent a syrup containing 65 per cent sugar was added to milk in the ratio of 2:3 (v/v). The mixture was again concentrated till the total solids were about 74 to 75 percent and the sugar content in the finished product was around 44 per cent. Condensed milk thus prepared was cooled and stored.

Storage of condensed milk: The glass tubes containing about 50 g of the finished product were sealed by the application of a number of coatings of melted paraffin wax around the cotton plug on the tubes. The condensed milk samples were stored at 37°C±1°C in an incubator upto 4 months. The quality of the product was assessed at 1-month interval.

Determination of viscosity: The viscosity of the condensed milk thus prepared was measured with Ferrantic Portable Viscometer[5]. Viscosity of goat milk samples before and after forewarming was determined by the capillary flow method using Ostwald Viscometer under a fixed pressure, at 30°±0.05°C. Total protein was estimated by Lowry method[6].

Estimation of proteose-peptone: Condensed milk was diluted 50 times. Five millilitre of the diluted sample was taken in a test tube and 0.5 ml of 10 per cent acetic acid and 0.5 ml of 1 N sodium acetate were added to it. After thorough mixing, it was kept in a water bath at 40°C for 20 min and centrifuged. The supernatant was used for the estimation of protein by Lowry method.

Estimation of non-protein nitrogen: Three ml of the casein free filtrate was mixed with equal volume of 16 per cent TCA and the mixture was shaken thoroughly and left for 30 min. It was filtered through Whatman No. 42 filter paper and the filtrate was used for the estimation of protein as described earlier.

Paper electrophoresis: One gram of condensed milk was diluted to 50 ml. Five millilitre of the diluted sample was taken in a test tube and casein was precipitated by adding 0.5 ml of 10 per cent acetic acid and 0.5 ml of 1 N sodium acetate. Casein was collected by centrifugation and washed three times with distilled water. Casein thus obtained was dissolved in urea-veronal buffer, pH 8.6 (urea-veronal buffer was prepared by dissolving 10 g urea in 100 ml veronal buffer, pH 8.6). Electrophoresis was carried out on an L.K.B. (Sweden) apparatus as described by Sabarwal and Ganguli[7]. The paper strips were scanned in a densitometer.

Results and Discussion

Level and distribution of protein in goat milk: The milk which was used for the manufacture of condensed milk was first analysed for different protein fractions. Results in Table I reveal that total protein in goat milk was of 3.23 g/100 ml milk. Casein constituted the major portion (73.76 per cent) of the total protein. The percent distribution of total protein into whey proteins, proteose-peptone and non-protein nitrogen fraction was 19.74, 4.95 and 1.57 per cent respectively. These results are in reasonably good agreement with those of Uusi-Rauva *et al*[8]. Whey proteins and non-protein nitrogen levels (Table I) agree well with the reported data of Nirmalan and Nair[9].

TABLE 1. LEVEL AND DISTRIBUTION OF PROTEIN IN GOAT MILK*

Constituents	g/100 ml milk		% of the total protein
	Range	Average	
Total protein	2.56–3.99	3.23	—
Casein	1.98–3.22	2.38	73.76
Whey proteins	0.57–0.77	0.64	19.74
Proteose-peptone	0.11–0.18	0.16	4.95
Non-protein N fraction	0.02–0.07	0.05	1.57

*Number of samples analysed were five.

Distribution of casein fractions as revealed by paper electrophoresis: The relative distribution of different fractions of casein in raw and heated milk was assessed by paper electrophoresis. It was observed that casein from raw milk samples resolved into two fractions, i.e. α-casein and β-casein, whereas a third fraction also appeared in forewarmed milk.

The data reported in Table 2 indicate that the concentration of β-casein is much higher as compared to α-casein. The relative proportion of β-and α-casein in raw milk was 63.45 and 36.54 per cent, respectively. On preheat treatment there was no change in the α-casein but concentration of β-casein was decreased from 63.54 to 58.86 per cent. The relative proportion of the new fractions which appeared on preheat treatment was 4.83 per cent. The higher level of β-casein than α-casein in goat milk irrespective of the status of milk, i.e. raw or forewarmed milk (Table 2), is in close agreement

TABLE 2. THE RELATIVE DISTRIBUTION OF DIFFERENT FRACTIONS OF CASEIN FROM RAW AND PREHEATED MILK AS REVEALED BY PAPER ELECTROPHORESIS

Nature of milk	Casein %					
	α-casein		β-casein		Unidentified fraction	
	range	Average	range	Average		
Raw milk	34.6 37.7	36.5	62.2 65.3	63.4	—	—
Preheated milk	35.3 37.2	36.3	58.8 58.8	58.8	3.90–5.7	4.8

TABLE 3. GROSS COMPOSITION OF CONDENSED MILK PREPARED FROM GOAT MILK

Sample No.	Total solids %	Total milk solids %	Sugar %
1	73.5	28.5	45.0
2	69.1	27.1	42.0
3	76.1	31.0	45.1
4	76.0	31.0	45.0
5	73.8	30.0	43.8

with the reported value of Singh and Ganguli[10], Hofman[11], Warner[12] and Prodanski and Petrow[13]. Zittle and Custer[14] also made similar observations.

Gross composition of condensed milk: The condensed goat milk thus prepared was analysed for its total solids, total milk solids and sugar contents. The results in Table 3 reveal batch to batch compositional variation. The total solids content varied from 69.10 to 76.10 per cent. Similarly the total milk solids and sugar were in the range of 27.10–31.10 and 42.0–45.0 per cent, respectively.

Viscosity of goat milk: With a view to assess the suitability of goat milk to utilize for sweetened condensed milk or evaporated milk, raw milk and milk heated at 15 lb pressure with no holding time was checked for its viscosity. Results in Table 4(*a*) indicate that there is no appreciable change in the viscosity of raw and forewarmed milk. The average viscosity of raw and forewarmed milk was observed to be 1.71 and 1.73 millipoises, respectively. During the preparation of evaporated milk from buffalo milk, Yadav[15] has found that if the forewarming temperature and time combination is kept at 120°C with no holding, there is very little rise in the viscosity of milk.

Viscosity of condensed milk during storage: Changes in the viscosity of condensed milk prepared from goat milk during storage at 37°C was also studied and the results are expressed in Table 4(*b*).

Observations recorded in Table 4(*b*) indicate that viscosity of condensed milk increased from 14.27 to 22.35 due to storage upto one month. On extended storage upto 4 months a considerable increase in the viscosity of the condensed milk samples was observed. Viscosity increased from 14.27 to 49.98 poises. Our results (Table 4*b*) concur well with the reported value of Yadav[15] for evaporated milk manufactured from cow and buffalo milk. He reported that there is a distinct increase in the viscosity of evaporated milk on storage at 37°C and on storage of evaporated milk at 4 to 6°C though the viscosity increased, the rate of such increase was much lower than that stored at 37°C. The increase in the viscosity of condensed milk might be due to the hydration of casein micelle during storage of the product. Aleshin *et al*[16]. studied the structural changes in proteins of condensed milk by electron microscope. They observed that the flaky appearance of denatured whey proteins in freshly prepared condensed milk becomes more compact on storage which result in the formation of net-like structures on binding with slightly loosened and deformed micelle. The interaction of active func-

TABLE 4*a*. VISCOSITY OF RAW AND PREHEATED GOAT MILK USED FOR CONDENSED MILK

Nature of samples	Viscosity (in milipoises)	
	Range	Average
Raw milk	1.56–1.83	1.71
Preheated milk	1.67–1.81	1.73

TABLE 4*b*. CHANGES IN VISCOSITY OF CONDENSED MILK DURING STORAGE

Storage period (months)	Viscosity (in poises)	
	Range	Average
0	9.57–23.17	14.27
1	10.96–34.15	22.35
2	21.32–62.47	37.37
3	24.99–64.14	42.76
4	31.65–71.63	49.98

TABLE 5. CHANGES IN THE PROTEINS DURING STORAGE OF CONDENSED MILK AS REVEALED BY PAPER ELECTROPHORESIS

Storage period (months)	∝-casein		β-casein		Fraction I		Fraction II	
	Range	Average	Range	Average	Range	Average	Range	Average
1	25.6–28.5	27.7	41.3–47.2	44.9	21.9–26.4	24.2	2.8–4.6	3.0
2	27.9–33.2	30.6	36.9–38.6	37.8	26.6–30.6	28.6	2.7–3.1	2.9
3	20.2–29.1	24.7	35.5–38.5	37.0	33.0–36.5	34.7	2.2–4.7	3.4
4	16.4–22.9	19.2	25.5–33.5	30.4	35.0–47.6	42.8	5.3–8.9	7.4

TABLE 6. CHANGE IN THE LEVEL AND DISTRIBUTION OF MILK PROTEINS DURING STORAGE OF CONDENSED MILK

Storage period (months)	Total protein %	Casein + Whey protein		Proteose-peptone		Non-protein N fractions	
		g/100 ml	% of total protein	g/100 ml	% of total protein	g/100 ml	% of total protein
0	10.01	9.48	94.70	0.23	2.26	0.30	3.01
1	10.01	9.35	93.40	0.15	1.49	0.50	4.99
2	9.95	8.93	89.74	0.39	3.91	0.62	6.23
3	9.98	8.49	85.07	0.69	6.91	0.80	8.01
4	9.98	8.16	81.76	0.92	9.22	0.91	9.00

tional groups results in the formation of the net-like protein structures and this might be the cause of gelation during storage of condensed milk.

Changes in the distribution of the protein fractions of condensed milk during storage: The status of casein fractions in the finished product stored at 37°C was assessed using electrophoretic technique. The perusal of Table 5 would indicate that on storage two new unidentified protein fractions (I and II) in order of increasing mobility appeared. The relative proportion of α-casein increased from 27.78 to 30.59 per cent during the second month of storage. During subsequent storage upto 4 months a gradual decrease in relative concentration (27.73 to 19.25 per cent) of α-casein was noticed. The relative proportion of β-casein also decreased from 44.90 to 30.48 per cent during storage upto 4 months. On the other hand, fraction I and fraction II showed a steady increase in its concentration during storage. The increase in case of fraction I was from 24.29 to 42.80 per cent and that in the fraction II from 3.05 to 7.44 per cent on storage upto 4 months.

Level and distribution of milk proteins during storage[2] Changes in the major milk proteins during the storage of the condensed milk were evaluated. The observations recorded in Table 6 clearly indicate that there is not much change in the level of total protein of condensed milk during storage. The casein and whey protein fraction showed decreases from 94.80 to 93.40 per cent during the first month of storage. On extended storage upto 4 months the decrease was from 94.70 to 81.76 per cent, thereby showing that this protein fraction is degraded to smaller protein fragments during storage.

Proteose-peptone content of condensed milk decreased from 2.26 to 1.49 per cent during the first month of storage, after which period it showed a marked increase. The increase in the concentration of proteose-peptone was much more sharp at 3 and 4 months of storage. At the end of 4 months the level of proteose-peptone was 9.22 per cent. Like proteose-peptone the non-protein nitrogen fraction also increased considerably with the storage period. It increased from 3.01 to 9.09 per cent during 4 months of storage. This also supports above contention that during storage the bigger molecular size proteins are broken down to smaller fractions.

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Production and Shelf Life of Spray-Dried *Srikhand* Powder

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After several trials on 150 litres of milk, spray-dried *Srikhand* powder was prepared from cow and buffalo milk. The product was gas packed and stored for 90 days at 30±1°C. The moisture content and volatile fatty acids in *Srikhand* powder during storage were more or less stable but there was a rise in free fat. Reconstitutability and flavour of both products were satisfactory for about 45 days. Thereafter storage of the buafflo *Srikhand* powder was less satisfactory.

Srikhand is prepared from Curd or dahi after draining out the water and blending with sugar, colouring matters and spices. Laxminarayana and Iya[1] compiled data on the types of fermented milks prepared in different parts of the world and indicated the organisms used with each type.

Although *dahi* is very popular in India, published data about it are meagre. Srinivasan and Anantakrishnan[2] have summarised the average composition of good quality *dahi* from cow and buffalo milk. An ICAR study on *dahi* reports[3] that market samples in west Bengal, U. P. and Panjab were characterised by good texture, sweet or mildly sour taste and pleasent aroma. Bhatia *et al*[4] have reported on the feasibility of producing good-quality dehydrated curd by drying.

Chakka was prepared by Ganguli *et al*[5] in the laboratory with approximately 60 per cent moisture and 22 per cent fat. Market samples of *chakka* showed wide variations in chemical composition. No information seems to have been published on the microbiological quality of *chakka* and *srikhand.*

Preparation of a reconstitutable *srikhand* powder from cow and buffalo milk and its keeping quality is reported in this paper.

Materials and Methods

Both cow and buffalo milk were obtained from the NDRI farm. In each trial 150 litres of milk were used. Standardisation to evolve the procedure was carried out with fresh skim milk in a cheese vat. The milk was pasteurised by heating to 71°C for 10 min and cooled to 28-29°C.

Hansen's L. F. starter culture (lactic fermentii, consisting of mixtures of different strains of *S. lactis*) at 1.0 per cent level was mixed throughly into the milk. The cultured milk was incubated at 25-28°C for 10-12 hr. The curd was collected in a muslin cloth and left hanging to enable drainage of whey. The total draining time was 8-10 hr to get a moisture content in the final base (or *chakka*) of about 60-65 per cent. Good quality sugar was added to the *chakka* at the rate of 18 kg per 100 kg and kneaded till a smooth body was obtained. The *srikhand* a slurry was homogenised in a single-stage homogeniser at room temperature at 100 kg/Cm^2 pressure. The homogenised slurry was adjusted to about 35 per cent total slids and spray dried at an inlet air temperature of 180-200°C and outlet temperature of 100°C. The *srikhand* powder after cooling was packed in tin containers of 75 g capacity with double nitrogen gas packing. The packed product was stored in an incubator at 30±1°C and removed for chemical and sensory evaluation at the initial stage and after 20, 45, 60, 75 and 90 days.

Chemical analysis of Srikhand and Srikhand powder: Samples of *dahi* and *chakka* were examined for fat, S.N.F., T.S. and acidity, pH, volatile fatty acids, free fat and peroxide value. The cans of *srikhand* powder opened at regular intervals were also examined physically. The dried product was reconstituted by adding 60-65 per cent hot water, and raising the temperature to about 60-70°C with constant stirring. When satisfactory consistency as indicated by viscous flow and body of the product was attained, the contents were transferred to the refrigerator for immediate cooling. After about 3-5 hr the reconstituted *srikhand* samples were examined for body, texture and flavour by a trained panel.

Milk, *dahi*, *chakka* and *srikhand* powder were analysed according to standard methods. The volatile fatty acids were determined by the method of Hempeniens and Liska[6] and peroxide value by the method of Smith[7]. Free fat was determined by the method of Hall and Hedrick[8], in which the product is extracted by gentle inversion in a graduated cylinder with light petroleum when only the fat unprotected by a membrane will be solubilised.

TABLE 1. COMPOSITION OF MILK AND DIFFERENT PRODUCTS

	Fat %	Moisture %	SNF %	Total solids %	Acidity %	pH	Volatile fatty acids[a]	Free fat[b]
Milk								
Cow	3.5	87.37	9.13	12.63	0.15	6.65	—	—
Buffalo	6.0	84.66	9.34	15.34	0.15	6.60	—	—
Chakka								
Cow	8.91	66.87	—	33.13	0.85	4.60	2.74	—
Buffalo	14.74	61.18	—	33.80	0.79	4.66	3.00	—
Srikhand powder								
Cow	22.88	3.49	—	96.51	0.24	4.60	6.16	12.89
Buffalo	32.98	2.87	—	97.12	0.26	4.70	6.48	21.84

a. Volatile fatty acids as ml of 0.1 N NaoH per 100g.
b. from fat as % total fat.

Results and Discussion

Analyses of standardised cow and buffalo milk used for the preparation of *srikhand* by way of *chakka* (drained *dahi*) are given in Table 1. *Chakka* from cow's milk had a fat percentage of 8.91 and from buffalo milk 14.74 per cent. Acidity of *chakka* from cow milk was 0.85 per cent and in buffalo milk 0.79 per cent. The volatile fatty acids, expressed as ml of 0.1 N NaoH per 100 g of *chakka* were 2.74 and 3.00 for products from buffalo and cow milk respectively.

The average fat percentages were 22.88 and 32.98 for *srikhand* powder prepared from cow and buffalo milk respectively. Acidity percentages were 0.24 and 0.26 respectively, and moisture contents 3.49 and 2.87 per cent. About 16 per cent of the volatile fatty acids were lost during drying of *chakka* to *srikhand* powder.

The free fat expressed as percentage of fat were 12.89 and 21.84 for cow and buffalo *srikhand* powders respectively. A trend towards an increase in fat free level with increase in the total fat content of dried *srikhand* was observed. Peroxide value of the fat was nil in all the batches of fresh *srikhand* powder. The average analytical values for spray-dried *srikhand* powder during storage at 30 ± 1 °C are tabulated in Table 2. Moisture contents remained more or less stable in the sealed tins as did the acidity. An increase in the amount of free fat (by 0.48 and 1.95 per cent respectively) was observed. This could happen through weakening the fat globule membranes during early stages of processing and drying, and rupture through expansion of dispersed air cells in the dried particles on storage. Volatile fatty acids tended to decrease slightly on storage of both products. Peroxide values showed no increase during this period.

The reconstitutability of stored *srikhand* powders and

TABLE 2. CHANGES ON STORAGE OF SPRAY-DRIED SRIKHAND POWDER

Storage period (days)	Fat %	Moisture %	SNF %	Total solids %	Acidity %	pH	Volatile fatty acids[a]	Free fat[b]
Cow milk product								
0	22.88	3.49	73.63	96.51	0.25	4.66	6.16	12.89
45	22.96	3.42	73.62	96.58	0.26	4.65	5.97	13.31
90	22.94	3.21	73.72	96.66	0.27	4.55	5.67	13.37
Buffalo milk product								
0	32.98	2.87	64.14	97.12	0.25	4.60	6.48	21.83
45	33.02	2.80	64.17	97.19	0.27	4.56	6.16	22.65
90	33.01	2.64	64.18	97.19	0.27	4.56	6.00	23.78

a. Volatile fatty acids as ml of 0.1N NaoH per 100g
b. from fat as % of total fat.

TABLE 3. PANEL EVALUATION OF PRODUCTS RECONSTITUTED FROM STORED, DRIED SRIKHAND POWDER

	Storage period		
	0 day	45 days	90 days
Reconstitution of powder			
Cow	Excellent	Good	Good
Buffalo	,,	,,	Fair
Flavour of reconstituted product			
Cow	8.5	8.1	7.0
Buffalo	8.2	7.5	5.8

a. Maximum grade point of 10.

the organoleptic quality of the products are presented in Table 3. Reconstitutabilities of fresh cow and buffalo *srikhand* powders were excellent, and this quality remained unaffected upto 30 days of storage. After 90 days of storage, buffalo *srikhand* powder reconstituted poorly. Flavour of the reconstituted product was acceptable upto about 45 days, but deterioration was rapid thereafter specially for buffalo *srikhand* powder.

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Utilization of Whey for Production of Microbial Protein and Lipid

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Three fungi viz. *P. frequentans*, *A. nidulans* and *Fusarium* N_{11} were grown in cheese whey for production of fungal protein and lipid. The above cultures were able to utilize the nutrients in deproteinized cheese whey and produce significant amount of biomass. The effect of supplementation at various concentrations of different carbon, nitrogen and growth factors on biomass, total protein and lipid were also studied. Glucose (7.5%), NH_4NO_3 (0.3%) and yeast extract (0.1%) had profound influence on growth and synthesis of protein and lipid. The biomass recovery (g /100 ml. medium) was highest in case of *P. frequentans* (2.482) followed by *A. nidulans* (2.440 g) and lowest in *Fusarium* N_{11} (2.100 g) under similar conditions. *P. frequentans* produced 39.81% of total protein and *A. nidulans gave* 36%. Lipid production was maximum in *Fusarium* N_{11} (38.56%), while it produced minimum amount of protein (16.62%). Analysis of fungal protein showed presence of all essential amino acids. GLC analysis of the methyl esters indicated the preponderance of C_{18} group of fatty acids.

The importance of whey as a high grade nutritive product in human nutrition has long been overlooked. Development of processes for the biological transformation of whey into microbial biomass, rich in protein, fat and vitamins, for use as a food supplement, would be of considerable significance. The present study has been undertaken to find out the feasibility of utilizing whey for production of microbial protein and fat under laboratory conditions using some selected mold strains.

Materials and Methods

The cultures used in the study included *Penicillium frequentans* obtained from Indian Agricultural Research Institute, New Delhi, and *Aspergillus nidulans* LC-1 and *Fusarium* N_{11} isolated at the National Dairy Research Institute, Karnal.

Maintenance of culture: The cultures were maintained on Potato dextrose agar (PDA) slants (pH 3.5-4.0) in screw capped test tubes at 30°C. After good growth

had been observed, cultures were stored in a refrigerator at 5°C. They were subcultured at monthly intervals.

Inoculum: Ninety six hour old slant cultures (vegetative mycelia) suspended in saline (growth from 2 slants suspended in 10 ml) were used as inoculum at 4 per cent level.

Incubation: After in cubation with mold cultures, 500 ml flasks containing 100 ml media were kept on a rotary shaker at 28±2°C and agitated for 96 hr.

Preparation of whey: Whey was collected from the cheese plant of experimental dairy. The pH of whey was adjusted between 5.0 and 5.2 and autoclaved for 10 min at 15 lb psi. The coagulated whey proteins were filtered through Whatman filter paper No. 42. The clear pale yellow protein free whey thus obtained was reautoclaved.

Composition of cheese whey: The cheese whey contained water, 93.0; lactose, 4.7; nitrogeneous matter, 0.9; ash, 0.7; fat, 0.3; and lactic acid, 0.25 per cent.

The basal medium for biomass production contained deproteinized whey, 100 ml; potassium chloride, 0.2 g; potassium dihydrogen phosphate, 0.5 g; and magnesium sulphate ($MgSo_4.7H_2O$), 0.1 g.

All ingredients were sterilized separately at 15 lb psi for 15 min. pH was adjusted to 4.5-5.0.

Effect of nitrogen: Ammonium nitrate (NH_4No_3), 0.3; urea, 0.1; and sodium nitrate ($NaNO_3$),0.3 per cent were supplemented to the basal medium as nitrogen sources. Glucose at various concentrations (2.5, 5.0 and 7.5 per cent) and molasses (5 per cent) were incorporated in to the basal medium as carbohydrate supplements. Yeast extract and malt extract were used at 0.1 per cent level each.

Harvesting of mycelium: The broth containing the mycelial mass was filtered and washed twice with 50 ml distilled water. The mycelial mass was pressed well between folds of filter paper and dried in a clean petridish at 80°C to constant weight.

Determination of protein: Total nitrogen was estimated by microkjeldahl method[1]. Total protein was calculated by multiplying the nitrogen content of mycelium by 6.25.

Determination of amino acid composition: Hydrolysis of protein was carried out according to the procedure described by Block[2]. Two dimensional paper chromatographic method[3] for separation of amino acids using solvent system, solvent I-Butanol; glacial acetic acid; water; 12:3:5 and solvent II-phenol: water: 4:1 was adopted.

Extraction and analysis of total lipids: Total lipids were extracted with chloroform:methanol according to the procedure described by Folch *et al*[4]. as modified by Dierkauf and Booij[5]. Lipids were finally dissolved in an aliquot of chloroform:methanol (2:1) mixture and stored at low temperature for chemical analysis. Identification of different classes of lipid were carried out by TLC on Silica gel-G (E. Merck) using petroleum ether: ethyl ether: acetic acid (90:10:1 v/v)[6].

Determination of fatty acid composition by GLC: Fatty acid methyl esters of the extracted lipid were prepared by the method of DeMan[7]. Esters were analysed by GLC using F and M model 609 hydrogen flame ionisation gas chromatograph. The peaks were measured by triangulation.

Results and Discussion

The data given on production of biomass, total protein and lipid (Table 1 to 4) are average values of 4 experiments generally given for the highest concentration of supplements.

Growth in deproteinized whey: The three molds were able to grow in deproteinized whey without any supplementation and the results are given in Table 1. *A. nidulans* gave maximum biomass (1.47 g/100 ml medium), *Fusarium* N_{11} produced highest amount of total lipid (31.14 per cent), while protein yield was maximum in *P. frequentans* (35.0 per cent).

Effect of various supplements: Data in Tables 2, 3 and 4 reveal that supplementation of deproteinized whey with different sources of carbon, nitrogen and growth factors had appreciable influence on biomass but little on total protein and lipid contents of the molds. Among the carbon sources used (Table 2) glucose at 7.5 per cent level had significant effect on overall growth. An increase in glucose concentration resulted in a proportionate increase in biomass, protein and lipid contents, higher the concentration better being the yield. Between 5.0 and 7.5 per cent glucose level there was not much difference as in lower concentrations. Ammonium nitrate was found to have some influence among nitrogen sources (Table 3) on growth and synthesis of protein and lipid. The presence of sodium nitrate and urea showed an irregular pattern of growth which was further reflected in the protein and

TABLE 1. GROWTH OF SELECTED MOLD STRAINS ON DEPROTEINIZED CHEESE WHEY

	A. nidulans		*Fusarium* N_{11}		*P. frequentans*	
	72 hr	96 hr	72 hr	96 hr	72 hr	96 hr
Mycelium wt (g)	0.99	1.47	0.92	1.24	0.91	1.11
Total protein (%)	30.75	30.75	13.12	13.12	35.00	35.00
Total fat (%)	9.70	10.04	30.26	31.14	5.06	6.80

Figures given are average values of four trials. Data are on dry wt basis.

Cultures were grown in 100 ml deproteinized cheese whey without any supplements in 500 ml flasks and kept at 28±2°C in a Shaker incubator.

TABLE 2. EFFECT OF CARBOHYDRATE ON BIOMASS, TOTAL PROTEIN AND LIPID CONTENT OF 3 MOLD STRAINS

Organism	Carbohydrate source	Concn %	Dry wt. of mycelium (g)	Total protein (% dry wt.)	Total lipid (% dry wt.)
A. nidulans	Glucose	2.5	1.82	34.61	10.25
		5.0	2.11	34.61	12.48
		7.5	2.11	35.00	12.20
	Molasses	5.0	2.12	33.35	12.17
Fusarium N_{11}	Glucose	2.5	1.78	13.12	35.10
		5.0	1.98	13.12	36.72
		7.5	2.01	14.00	38.00
	Molasses	5.0	1.92	14.85	36.00
P. frequentans	Glucose	2.5	1.76	37.62	6.15
		5.0	2.12	38.80	7.14
		7.5	2.13	38.75	8.80
	Molasses	5.0	2.09	38.56	7.16

Figures are average of four experiments.

Cultures were grown in 100 ml media in 500 ml flasks containing 0.3% $NaNO_3$ and kept for 96 hr in a shaker incubator at $28 \pm 2°C$.

TABLE 3. EFFECT OF DIFFERENT NITROGEN SOURCES ON BIOMASS, TOTAL PROTEIN AND FAT

Organism	Nitrogen source	Concn (%)	Dry. wt. of mycelium (g)	Total protein (% dry wt.)	Total fat (% dry wt.)
A. nidulans	$NaNO_3$	0.3	2.11	34.61	12.48
	NH_4NO_3	0.3	2.44	35.00	16.90
	Urea	0.1	1.67	34.13	13.51
Fusarium N_{11}	$NaNO_3$	0.3	1.98	13.12	36.72
	NH_4NO_3	0.3	2.02	16.62	38.56
	Urea	0.1	1.81	16.62	38.54
P. frequentans	$NaNO_3$	0.3	2.12	38.80	7.14
	NH_4NO_3	0.3	2.48	38.50	10.53
	Urea	0.1	1.71	38.50	9.31

Figures are average of four experiments.

Cultures were grown in 100 ml media in 500 ml flaks containing 5% glucose and kept for 96 hr in a shaker incubator at $28 \pm 2°C$.

TABLE 4. EFFECT OF SUPPLEMENTATION OF GROWTH FACTORS ON BIOMASS, TOTAL PROTEIN AND FAT

Organism	Growth factor source	Concn (%)	Dry wt. of mycelium (g)	Total protein (% dry wt.)	Total fat (% dry wt.)
A. nidulans	Yeast extract	0.1	2.28	36.00	15.72
	Malt extract	0.1	2.08	32.69	13.12
Fusarium N_{11}	Yeast extract	0.1	2.10	15.75	37.48
	Malt extract	0.1	1.95	14.86	37.33
P. frequentans	Yeast extract	0.1	2.21	39.81	10.08
	Malt extract	0.1	2.13	38.80	8.69

Figures are average of 4 trials.

Cultures were grown in 100 ml media in 500 ml flasks containing 5% glucose and 0.3% $NaNO_3$ and incubated for 96 hr in a shaker at $28 \pm 2°C$.

TABLE 5. AMINO ACIDS IN SELECTED FUNGAL PROTEINS

Essential amino acid	*A. nidulans*	*Fusarium* N_{11}	*P. frequentans*
Isoleucine	+ + +	+ +	+ + +
Leucine	+ + +	+ +	+ + +
Lysine	+ + +	+	+ + +
Methionine	+ +	+ +	+ + +
Phenyl alanine	+ + +	+ +	+ + +
Threonine	—	+ +	+ +
Tryptophan	—	—	—
Valine	+ +	+ +	+ + +

+ = Presence and intensity of spots.
– = Absent.

lipid contents. The adverse effect of urea noticed in this study may be due to an increase in pH because of the release of ammonia and this might have exceeded the rate at which it was utilised by the mycelium. Yeast extract was found to have significant effect on biomass, protein and lipid contents (Table 4). Yeast extract is known to contain energy rich phosphates, co-factors and nucleotide bases in addition to compounds of vitamin B-complex group, and hence the enhanced growth.

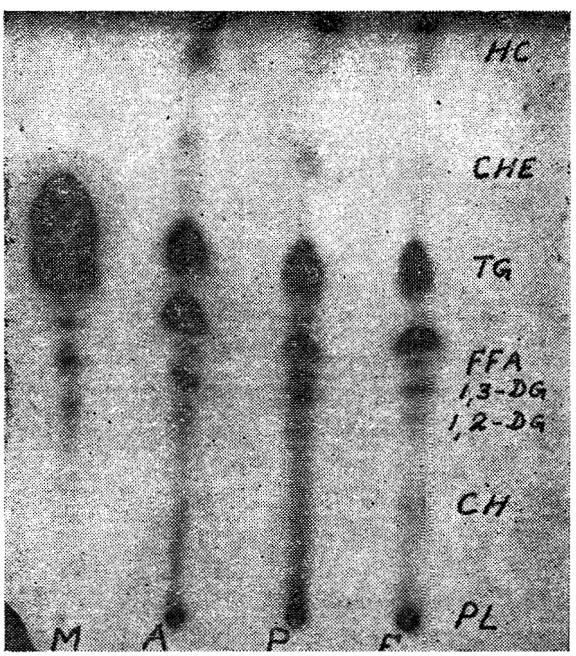

Fig. 1. Thin layer chromatogram showing lipid pattern of selected fungi.

M = Milk lipid; A = *A. nidulans;* P = *P. frequentans;* F = *Fusarium* N_{11}; PL = Phospholipid; Ch = Cholesterol; 1,2-DG = 1, 2 Diglyceride; 1,3-DG = 1, 3-Diglyceride; FFA = Free fatty acid; TG = Triglyceride; CHE = Cholesteryl ester; and HC = Hydrocarbon.

Biomass production: The biomass obtained from *P. frequentans* (2.48 g/100 ml medium) was the highest among organisms studied. Rattan Chand *et al*[8]. reported that *P. frequentans* gave highest yield of biomass (806 mg/50 ml media) out of 5 molds screened. *A. nidulans* gave 2.44 g followed by *Fusarium* N_{11} (2.10 g) under similar conditions.

Lipid: Fusarium N_{11} produced the highest amount (38.56 per cent) of lipid ranging from 31.0 to 38.0 per cent under different experimental conditions. Damm[9] reported 50 per cent lipid from *Fusarium* sp. grown on sulphite waste liquor containing 4 per cent carbohydrate. *Fusarium lycopersicum* was shown to produce 30.23 to 40.10 per cent lipid as observed by Rattan Chand *et al*[8]. *A. nidulans* produced 16.90 per cent (10-16 per cent) lipid while *P. frequentans* gave lowest yield (10.53 per cent) of lipid.

Protein: P. frequentans gave the highest (39.81 per cent) amount of protein. Stokes and Gunness[10] showed 38.4 per cent protein in *P. notatum* while Pruess *et al*[11]. reported 43.7 per cent in *P. aurantio-brunneum. A. nidulans* produced 36 per cent of protein ranging from 30 to 36 per cent while *Fusarium* N_{11} gave significantly low (16.62 per cent) protein.

Amino acid composition: The three mold strains contain approximately equal number of amino acids (Table 5). It is seen that all essential amino acids which determine the quality of protein, are present in fairly good concentrations. A close appraisal of the data

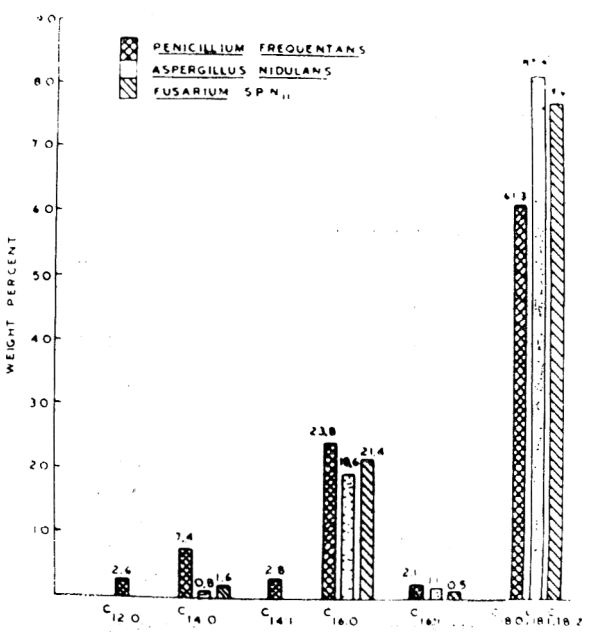

Fig. 2 Fatty acids of selected fungi.

C_{12} = Lauric acid; $C_{14:0}$ = Myristic acid; $C_{14:1}$ = Myristoleic acid; C_{16} = Palmitic acid; $C_{16:1}$ = Palmitoleic acid; $C_{18:0}$ = Stearic acid; $C_{18:1}$ = Oleic acid; $C_{18:2}$ = Linoelic acid; and $C_{18:3}$ = Linolenic acid.

reveal that methionine, valine, leucine, isoleucine and phenyl alanine are in higher concentrations. Lysine and arginine follow the same trend both in *P. frequentans* and *A. nidulans* but in *Fusarium* N_{11} these amino acids are in lower amounts. *P. frequentans* and *Fusarium* N_{11} contain equal amount of threonine where as *A. nidulans* is conspicuous by its absence. Tryptophan is absent in all chromatograms because of acid hydrolysis.

Lipid pattern and fatty acid composition: Lipid pattern of the extracted fat is shown in Fig. 1. Triglycerides are the major constituents followed by phospholipids and partial glycerides. Cholestero land cholesteryl esters and free fatty acids are found to be in lower concentrations.

GLC analysis of methyl esters showed the presence of relatively higher number of fatty acids (Fig. 2) in *P. frequentans*. Absence of fatty acids 12 and 14:1 and lower concentrations of 14:0 and 16:1 were reported by Robert Shaw[12]. The absence of these fatty acids may be traced to lack of definite enzyme systems, which can be correlated with the genetic constitution of the organisms. $C_{16:0}$ and C_{18} groups were in relatively higher quantities in all cases. *A. nidulans* gave the highest amount (81.4 per cent) of C_{18} acids.

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Studies on Milk Clotting enzyme from *Bacillus megaterium* K-40. I. Effect of Some Nutrients on enzyme Production

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The effect of certain nutrients on the production of milk clotting enzyme by *Bacillus megaterium* K-40 was studied. Maximum enzyme production was observed in 5% wheat bran medium after 48 hr of incubation at 37°C. Of the various organic supplements added to this basal medium, incorporation of only 1% molasses resulted in about 82% increase in the enzyme level at the end of 36 hrs. Addition of skim milk at 25% level also slightly increased the enzyme production. On the other hand, whey corn steep liquor, yeast extract, malt extract and certain partial hydrolysates of protein were either inhibitory or had no effect on the production of the enzyme. Various sugars incorporated into the basal medium, proved inhibitory to the enzyme elaboration. Similarly many inorganic salts except $CaCl_2$ adversely affected the enzyme production.

Shortage of animal rennet and its high price in the world market has necessitated a vigorous search for the development and production of rennet from microbial sources which serve as a substitute for the animal rennet[1-4]. Microbial rennet substitutes if produced indigenously will cut imports on calf rennet. Since the production of enzymes by microorganisms is influenced by various factors, especially the cultural and environmental conditions, optimization of these parameters is a prerequisite not only for commercial production but also for any meaningful basic study on the enzymes. Factors affecting the production of milk clotting enzymes by bacteria have been studied by various workers[5-10]. Earlier work in our laboratory has shown that bacterial rennet isolated from a few selected strains of *Bacillus subtilis*, *B. cereus* and *B. megaterium* could be used as animal rennet substitutes for cheese making[11]. The present report, as the first phase of our studies on milk

clotting enzyme from *B. megaterium* K-40, deals with the effect of some nutrients on the enzyme production.

Materials and Methods

Cultivation of the organism: The organism, *Bacillus megaterium* K-40, maintained as soil culture was transferred to Tryptone Dextrose Yeast Extract Agar slants and was incubated at 37°C for 24 hr. The growth from the slant was washed with 6 ml of sterile normal saline solution and the suspension was inoculated at 2 per cent level into 500 ml Erlenmeyer flasks containing 100 ml of 5 per cent wheat bran in distilled water (basal medium). The flasks were incubated on rotary shaker (about 200 rpm) upto 72 hr at 37°C and the milk clotting activity was assayed at regular intervals.

Assay of milk clotting activity: Assay method for the enzyme activity was the same as described by Srinivasan *et al.*[7], but was carried out at 37°C using 12 per cent reconstituted skim milk containing 0.01 M $CaCl_2$. One unit of enzyme is defined as the amount of enzyme required to clot 10 ml of reconstituted skim milk at 37°C in 60 sec.

Nutritional supplements: Various organic and inorganic supplements incorporated individually in the basal medium (5 per cent wheat bran) for studying their effect on the enzyme production were: Whey Powder (1 to 8 per cent); corn steep liquor (1, 3, and 5 per cent); molasses (1 to 5 per cent); skim milk (25, 50, and 100 per cent v/v); yeast extract, malt extract, peptone, proteose-peptone and tryptone at 0.5 and 1.0 per cent level; 0.5 per cent of glucose, fructose, lactose, sucrose and maltose; $CaCl_2$ (0.5 and 1.0 per cent); ammonium phosphate and phosphate mixture ($KH_2PO_4 + K_2HPO_4$) at 0.1 per cent level; ammonium sulfate (1.0 per cent), and salt mixture containing 0.1 per cent ammonium sulfate, 0.05 per cent KH_2PO_4 and 0.05 per cent $MgSO_4$ at 0.17 per cent level.

Results and Discussion

Production of milk clotting enzyme in wheat bran medium: Production of milk clotting enzyme from *B. megaterium* K-40 was initially studied in medium containing 1 to 8 per cent wheat bran and the activity assayed after 24, 48 and 72 hr of incubation on a rotatory shaker at 37°C. It may be observed from Table 1 that maximum production occurred in 5 per cent wheat bran at the end of 48 hrs as also reported by Dudani and coworkers[11] for 5 different strains of *B. subtilis*. Decline in enzyme activity was observed on further incubation. The organism failed to produce any milk clotting activity in 1 and 2 per cent wheat bran. For all subsequent studies therefore, 5 per cent wheat bran was used as the basal medium which was fortified with different organic and inorganic supplements.

TABLE 1. EFFECT OF WHEAT BRAN CONCENTRATION ON THE, PRODUCTION OF MILK CLOTTING ENZYME

Concn of wheat bran (% w/v)	Milk clotting activity (units/ml) at different Incubation period		
	24 hr	48 hr	72 hr
1	<0.001	<0.001	<0.001
2	<0.001	<0.001	<0.001
3	0.21	0.26	0.25
4	0.30	0.82	0.97
5	0.43	1.11	1.09
6	0.46	0.83	0.43
7	0.41	0.63	1.00
8	0.26	0.46	0.62

Effect of organic supplements on enzyme production: Influence of various organic supplements to the basal medium on the elaboration of milk clotting enzyme by *B. megaterium* K-40 is presented in Table 2. Whey when tested singly or in combination with 5 per cent wheat bran failed to produce any appreciable quantity of milk clotting enzyme even after 72 hr of incubation. This observation is in accordance with that of Srinivasan *et al*[7]. While no enzyme was elaborated in 1, 3 and 5 per cent corn steep liquor singly, the activity observed in the basal medium supplemented with corn steep liquor (Table 2) was much lower than that of control. Sannabhadti[12] also observed a decrease in the amount of milk clotting enzyme produced by *Absidia ramosa* in corn steep liquor.

Molasses when incorporated into 5 per cent wheat bran showed a positive influence on the enzyme production by the test organism. Fortification of the basal medium with 1 per cent molasses enhanced the clotting activity by 82 per cent. Further increase in the concentration of molasses in the medium had an adverse effect. Elaboration of the enzyme in the basal medium alone and after fortification with 1 per cent molasses, as function of time, is given in Fig. 1. While maximum activity (1.14 units/ml) was obtained in basal medium after 48 hr, the incubation period needed to achieve the maximum activity (1.82 units/ml) in the fortified medium was reduced to 36 hrs. On further incubation, whereas 66.3 per cent of the maximum activity was still retained in the wheat bran-molasses medium after 96 hrs, only 17.5 per cent of the maximum activity was detected in the case of basal medium. These findings are supported by the earlier study of Sannabhadti[12] in the case of *A. ramosa*.

Other organic supplements such as yeast extract, malt extract and partial hydrolysates of proteins like, peptone, proteose-peptone and tryptone were found to be inhibi-

TABLE 2. EFFECT OF DIFFERENT ORGANIC SUPPLEMENTS ON THE PRODUCTION OF MILK CLOTTING ENZYME

Supplement to Basal medium	Concn. (% w/v)	Milk clotting activity (units/ml) at different Incubation periods				
		24 hr	36 hr	48 hr	60 hr	72 hr
None	—	0.60	1.00	1.14	0.85	0.60
Molasses	1.0	0.54	1.82	1.58	1.39	1.28
	3.0	0.63	0.79	0.98	0.97	0.91
	5.0	0.30	0.56	0.71	0.86	0.82
Corn Steep—	1.0	0.50	0.58	0.71	0.60	0.43
Liquor	3.0	0.18	0.24	0.19	<0.001	<0.001
	5.0	0.20	0.32	0.40	0.22	<0.001
Skim milk	25% (v/v)	0.24	0.32	0.52	0.86	1.67
	50% „	0.12	0.29	0.32	0.35	0.32
	100% „	0.10	0.12	0.13	0.10	0.22
Yeast Extract	0.5	0.58	0.76	0.72	0.67	0.54
	1.0	0.50	0.88	0.71	0.63	0.50
Malt extract	0.5	0.35	0.41	0.75	0.65	0.63
	1.0	0.30	0.44	0.70	0.71	0.62
Peptone	0.5	0.20	0.29	0.31	0.35	0.51
	1.0	<0.001	0.16	0.31	0.36	0.35
Proteose—	0.5	0.21	0.35	0.43	0.73	0.81
Peptone	1.0	0.19	0.29	0.24	0.41	0.57
Tryptone	0.5	0.17	0.26	0.40	0.50	0.85
	1.0	0.29	0.32	0.28	0.56	0.40

tory for enzyme production. Srinivasan *et al*[7] also reported a decrease in the amount of enzyme production by 2 strains of *B. subtilis* when partial or complete protein hydrolysate products were incorporated into casein broth.

Unlike in the case of *B. subtilis*[7], incorporation of skim milk at 25 per cent level increased the enzyme production, from 1.14 units/ml after 48 hr incubation in basal medium to 1.67 units/ml at the end of 72 hr by *B. megaterium* K-40. However, the increase was less than that caused by 1 per cent molasses after only 36 hr incubation. A similar stimulation by skim milk in the milk clotting enzyme production by *A. ramosa* was observed by Sannabhadti[12].

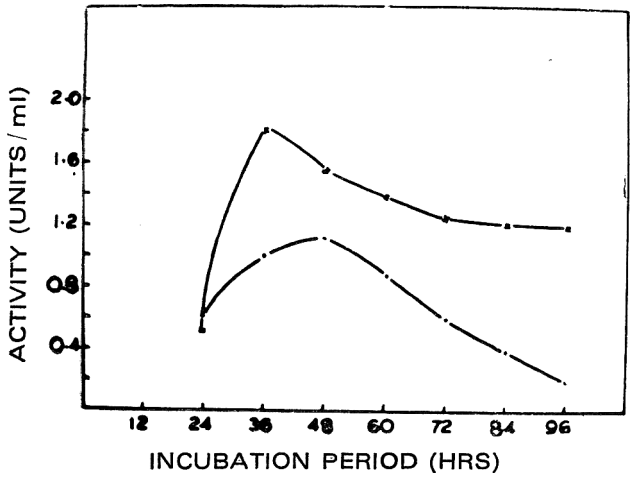

Fig. 1. Effect of molasses on the production of milk clotting enzyme by *B. megaterium* K-40.

.——. 5% Wheat bran; ×——× 5% Wheat bran+1% molasses.

Effect of sugars on enzyme production: Influence of fermentable sugars on enzyme elaboration was also studied by supplementing the basal medium with glucose, fructose, lactose, sucrose and maltose at 0.5 per cent level. It may be clearly seen from the results presented in Table 3 that all the sugars were inhibitory for the

TABLE 3. EFFECT OF SUGARS ON THE PRODUCTION OF MILK CLOTTING ENZYME

Supplement to Basal medium Source	Concn. (% w/v)	Milk clotting activity (units/ml) at different incubation periods				
		24 hr	36 hr	48 hr	60 hr	72 hr
None	—	0.60	1.00	1.14	0.85	0.60
Fructose	0.5	0.35	0.50	0.60	0.59	0.71
Sucrose	0.5	0.50	0.38	0.45	0.51	0.32
Lactose	0.5	0.35	0.42	0.75	0.77	0.62
Maltose	0.5	0.67	0.90	0.86	0.92	1.00

TABLE 4. EFFECT OF INORGANIC SALTS ON THE PRODUCTION OF MILK CLOTTING ENZYME

Salt added to Basal Medium Source	Concn. (% w/v)	Milk clotting activity (units/ml) at different incubation periods				
		24 hr	36 hr	48 hr	60 hr	72 hr
None	—	0.92	1.02	1.09	0.92	0.55
$(NH_4)_2SO_4$	1.0	0.35	0.41	0.46	0.36	<0.001
$(NH_4)_3PO_4$	0.1	0.29	0.29	0.34	0.41	0.46
$K_2HPO_4+KH_2PO_4$(0.05% each)	0.1	0.26	0.35	0.36	0.47	0.69
Salt mixture£	0.17	0.14	0.28	0.40	0.63	0.57
$CaCl_2$	0.5	0.65	0.82	0.71	0.83	0.63
	1.0	0.69	0.95	0.97	1.09	0.77

£Salt mixture consisted of 0.1% $(NH_4)_2SO_4$, 0.05% KH_2PO_4 and 0.02% $MgSO_4$.

enzyme production, the extent of inhibition varying with the particular sugar added. Maltose had the least adverse effect.

Effect of inorganic salts on enzyme production: Except $CaCl_2$, all the other inorganic supplements tested were observed to be inhibitory to the milk clotting enzyme production by the test organism (Table 4). While $CaCl_2$ at 0.5 per cent level was also slightly inhibitory, with 1 per cent $CaCl_2$ incorporated in the basal medium, 1.09 units/ml of enzyme activity was obtained after 60 hrs of incubation which is the same as that of control after 48 hr. Similar reports were made by Srinivasan *et al*[7] with two strains of *B. subtilis*.

Among the various organic and inorganic nutritional supplements studied for their effect on the production of milk clotting enzyme from *B. megaterium* K-40, incorporation of only 1 per cent molasses resulted in any significant increase in the enzyme activity over that obtainable in the 5 per cent wheat bran basal medium. The wheat bran-molasses medium has the added advantage of achieving about one and a half fold increase in the maximum activity with a reduction in the production time by 12 hr.

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Studies on Milk Clotting Enzyme from *Bacillus megaterium* K-40. II. Effect of Some Environmental Factors on Enzyme Production

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Effect of environmental factors like pH, temperature and agitation were studied to optimize conditions for the production of milk clotting enzyme from *Bacillus megaterium* K-40 using wheat bran-molasses as the growth and production medium. No enzyme production was observed at 45°C or at pH 4.5-5.5. Stationary cultures also failed to exhibit any significant enzyme production. The milk clotting enzyme was optimally produced in the post exponential growth phase of the organism in shake flask cultures, at the initial pH 5.8 of the medium when incubated at 37°C for 12 hr and subsequently at 30°C for an additional period of 24 hr.

In recent years, the feasibility of bacterial milk clotting enzymes as substitutes for the calf rennet has been investigated by various workers[1-6]. Srinivasan *et al*[7] isolated and examined a number of aerobic spore forming bacteria for the production of milk clotting enzyme and explored the possibility of preparing cheddar cheese by using this enzyme. Studies on the influence of different cultural and environmental factors for the optimal production of bacterial milk clotting enzyme have been conducted by many workers[8-10]. Our earlier work[11] as the first phase of the studies on the milk clotting enzyme from *Bacillus megaterium* K-40, related to the effect of some nutrients on the enzyme production. The present communication on optimising conditions for production of the milk clotting enzyme reports on the influence of certain environmental factors.

Materials and Methods

The details of cultivation of *Bacillus megaterium* K-40 for the production of milk clotting enzyme and its method of assay are the same as given in our earlier communication[11]. The effect of different environmental factors namely, initial pH of the medium, temperature and time of incubation, and agitation through shaking was studied in the wheat bran-molasses medium (5 per cent wheat bran and 1 per cent molasses) which was found suitable in our earlier experiments for the production of milk clotting enzyme. All the experiments were carried out in triplicate and the results reported are the mean values.

Results and Discussion

Effect of initial pH of the medium: Enzyme production by *B. megaterium* K-40 was studied on wheat bran-molasses medium initially adjusted to pH values ranging from 4.5 to 8.0 The maximum activity (1.09 unit/ml) was obtained in the medium adjusted to pH 6.0, which compared well with the peak activity of 1.11 units/ml in the unadjusted control (pH 5.8) after 36 hr incubation at 37°C on a rotary shaker (Table 1). While further increase in the initial pH resulted in decreased enzyme production, significant activity was noted at pH 7.5 also. No milk clotting activity was detected in media with initial pH adjusted between 4.5 and 5.5. The optimum pH for the enzyme production has been reported to be 7.0 for another strain of *B. megaterium*[10] and 4.0 to 7.0 for the fungal enzyme by *Absidia ramosa*[12].

Effect of temperature and period of incubation: Three sets of flasks with wheat bran-molasses medium (pH 5.8) were inoculated with the test organism at 2 per cent level and incubated on a rotary shaker at 30° and 37°C, and

Table 1. Effect of initial pH of the medium on the production of milk clotting enzyme

Initial pH	Milk clotting activity (units/ml) at different incubation					
	12	24	36	48	60	72
5.8	0.43	0.69	1.11	0.95	0.75	0.50
6.0	0.14	0.67	1.09	0.67	0.85	0.67
6.5	0.17	0.67	0.86	0.62	0.60	0.55
7.0	0.18	0.43	0.57	0.55	0.51	0.40
7.5	0.26	0.77	1.05	1.00	0.98	0.71
8.0	0.27	0.67	0.86	0.76	0.69	0.60

TABLE 2. EFFECT OF INCUBATION TEMPERATURE ON THE PRODUCTION OF MILK CLOTTING ENZYME

Temp. (°C)		Milk clotting activity (units/ml) at different incubation periods					
		12 hr	24 hr	36 hr	48 hr	60 hr	72 hr
37		0.41	0.54	1.82	1.58	1.45	1.28
30	I	<0.001	1.18	3.00	4.29	4.00	3.53
	II*	0.37	1.28	4.29	3.75	2.40	1.50

*Incubation at 37°C upto 12 hr and subsequently at 30°C.

45°C respectively. The milk clotting activity was assayed at every 12 hr interval upto 72 hr. No enzyme activity was detected in cultures incubated at 45°C. It may be noted from the data presented in Table 2 that the level of enzyme activity increased from 1.82 to 4.29 units/ml when the incubation temperature was changed from 37° to 30°C. However, the maximum enzyme production at 30°C was achieved after 48 hr as against 36 hr when incubated at 37°C. No enzyme activity could be assayed after 12 hr of incubation at 30°C whereas 0.41 units/ml of enzyme was produced at 37°C. This may likely be due to an increased lag period in the growth phase of the organism at the lower temperature. To reduce the initial lag period and to produce a considerable amount of cell mass, the cultures were initially incubated at 37°C for 12 hr and subsequently transferred to 30°C. This resulted in achieving the maximum level (4.29 units/ml) of enzyme production after a total period of 36 hr. These observations are in agreement with the findings of El-Sadak *et al.*[10] for a strain of *B. megaterium* and that of Sannabhadti[12] for *A. ramosa.*

Effect of shaking: Replicates of culture flasks with wheat bran-molasses medium (pH 5.8) were incubated initially at 37°C for 12 hr and subsequently at 30°C with continuous or intermittent shaking (for 15 min after every 12 hr) on a rotary shaker, and without any shaking.

TABLE 3. EFFECT OF SHAKING ON THE PRODUCTION OF MILK, CLOTTING ENZYME

	Milk clotting activity (units/ml) at diff. time (hr)							
	12	24	36	48	60	72	84	96
Continuous shaking	0.29	1.46	3.00	2.86	2.22	1.71	1.00	0.95
Intermittent shaking	<0.001	0.20	0.31	1.09	1.43	1.36	1.00	0.71
Without shaking	<0.001	<0.001	<0.001	<0.001	0.10	0.13	0.43	0.92

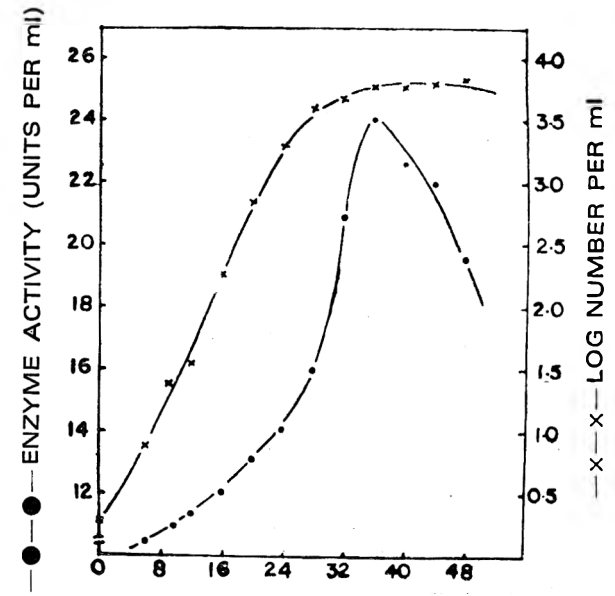

Fig. 1. Correlation between total cell count and production of milk clotting enzyme from *B. megaterium* K-40.

The culture was grown in wheat bran-molasses medium (pH 5.8) initially at 37°C for 12 hr and subsequently at 30°C on a rotary shaker.

.——., 5% wheat bran; ×——×, 5% wheat bran+1% molasses

The milk clotting activity in different flasks was assayed at 12 hr interval upto 96 hr. The data recorded in Table 3 show that the enzyme production in culture flasks without any shaking was comparatively low and could be detected only after 60 hr. Whereas maximum clotting activity (1.43 units/ml) was observed after 60 hr in the case of intermittent shaking, continuous shaking in the rotary shaker resulted in an enzyme activity of 3.0 units/ml after 36 hr. Others[8,10] however, observed an increased enzyme activity in cultures incubated with intermittent shaking.

Relationship between growth and enzyme production: It may be seen from the Fig. 1 that the production of milk clotting enzyme by *B. megaterium* K-40, under optimal conditions, was initiated in the early logarithmic growth phase, with a steep increase in activity in the postexponential growth phase, and sharply declined as the culture entered the stationary phase. These results are in agreement with those of Rao and Mathur[13] who reported the maximum rennet production in the logarithmic growth phase of *B. subtilis* K-26 in a 10 litre fermentor. Similar findings were also reported on the production of extracellular protease by a *Bacillus* sp[14,15].

It may be concluded from the results obtained in this study that the milk clotting enzyme from *Bacillus megaterium* K-40 is optimally produced in wheat bran-molasses medium at pH 5.8 when incubated on a rotary shaker initially at 37°C for 12 hr and subsequently at 30°C for 24 hr.

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Characteristics of *Roti, Dosa* and Vermicelli from Maize, Sorghum and Bajra

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Maize flour required more water for making dough for *roti* than sorghum, bajra or wheat flour. Maize *roti* was soft and less chewy than *roti* from the other grains, particularly after keeping for 6-8 hr. Moisture in the baked *roti* was also highest for maize. When *Dosa* blends of different grains with black gram (*Phaseolus mungo*) were made, maize did not give a satisfactory *dosa*. At equal slurry concentrations, maize batter had higher cold paste viscosity and lower hot paste viscosity as compared with the other grain flours. Sorghum gave *dosa* with good consumer acceptance. Vermicelli made from maize, sorghum and bajra flours completely disintegrated upon cooking. However, steaming maize and sorghum vermicelli strands for 20-30 min prior to drying conferred sufficient strength for better retention of shape during cooking.

Wheat and rice are normally used for making *roti** or *dosa** and vermicelli (*Savia*). Maize (*Zea mays*), sorghum (*Sorghum vulgaris*) and bajra (*Pennisetum typhoideum*) are not used widely for these purposes because of the coarse outer husk which is responsible for their relatively poor acceptability and palatability. Methods for dehusking these grains have been standardised at this Institute[1] and it has been shown that these pearled grains could be used for making many acceptable dishes normally prepared from rice and wheat.

The present study was undertaken to investigate the characteristics of *roti, dosa* and vermicelli prepared from maize, sorghum and millet flours.

Materials and Methods

Locally available hybrid sorghum ('CSH-5), white maize ('Ganga-S'), hybrid yellow maize and bajra ('HB-3') were used in these studies. Wholemeal flours were prepared by grinding in a plate grinder and -40 B.S. mesh flour fractions were used to prepare *dosa*, while -60 mesh flour was used for *roti* and vermicelli. Flour of -60 mesh from rice, black gram *dhal* (*Phaseolus mungo*), wheat and dehusked varagu (*Paspalum scrobiculatum*) were used for comparison.

Roti: As the millet grains lack gluten, the dough was made by using boiling water to give some adhesiveness during rolling of *roti*. Water needed to make the dough, the time of baking and the eating quality of the

**Roti* is a thin pancake from a dough, while *Dosa* is made from a thinner batter. Both are baked on a hot plate.

baked *roti* were determined. Boiling water was added in instalments to 100 g flour and throughly mixed each time before adding next lot of water. Minimum water needed to give a dough of consistency suitable for rolling it to a *roti*/chapati was determined. Dough (25 g) was rolled to definite size (15 cm diameter) and put on a hot pan until it was baked. To study the eating quality of the *roti*, a known amount (8 g each) of baked *roti* was judged by 10 persons (Table 1) and the number of mastication or chewing needed to swallow the *roti* was determined[2]. Moisture content and the eating quality were also determined in *rotis* immediately after making and also after being kept for upto 24 hr period in the following conditions viz., (*i*) closed container, (*ii*) open container, (*iii*) open container after sprinkling 6 per cent extra moisture on the baked *roti* or *chapati*, (*iv*) 300 gauge polythene bags and (*v*) rolled in wet cloth.

Dosa: The *dosa* flour blend contained 65 g maize/sorghum/millet flour, 20 g wheat flour and 15 g black gram flour. Calcium carbonate (2 per cent) and citric acid (2 per cent) were used as leavening agents. The mixes were added to 300 ml of water in case of maize and 250 ml of water each in case of sorghum and bajra for making the *dosa* batter. The culinary qualities of the *dosa* such as ease of baking, turnability on the pan and eating quality were noted. Cold paste viscosity of the batter from the *dosa* mix and individual milled flours at different slurry concentrations was determined using a Brookfield viscometer. *Dosa* mixes containing rice or wheat were used for comparison. Hot paste characteristics of different *dosa* batters were determined in a Brabender Visco-Amylograph using a 10 per cent slurry[3].

Vermicelli: Boiling water (130 ml) was used to make the dough from 100 g millet flour. The dough was extruded through a vermicelli press having 0.2 mm aperture die. The vermicelli strands were then steamed for 0, 10, 20 and 30 min and then air dried at 60°C. To determine the cooking quality of the dried vermicelli, a 10 g sample was taken in a cylindrical wire basket of 5 in. height and 1 in. diameter, immersed in a beaker containing 100 ml boiling water, and cooked for 5 min. The percentage of solids leached into the cooking water was determined[4] by drying to constant weight at 110°C.

TABLE 1. CHARACTERISTICS OF *ROTI* BASED ON DIFFERENT FLOURS

	Water needed for making dough (ml/100 g)	Time of baking (sec)	Moisture in *Roti* after baking (%)	Chewing counts	
				Immediately after baking	2 hr after baking
Maize	110	120	40.2	31± 6.9	36± 8.7
Sorghum	90	105	36.2	38± 7.7	50±10.0
Bajra	85	105	32.2	37± 6.9	40±10.7
Wheat	65	90	31.0	40±11.3	50± 6.2
Wheat + Maize (70+30)	80	110	35.8	34± 8.6	—
Wheat + Sorghum (70+30)	65	100	30.4	36± 7.9	—

Results and Discussion

Roti: Water needed for making dough, baking time and quality of baked *rotis* are presented in Table 1. Maize required more water for making the dough and

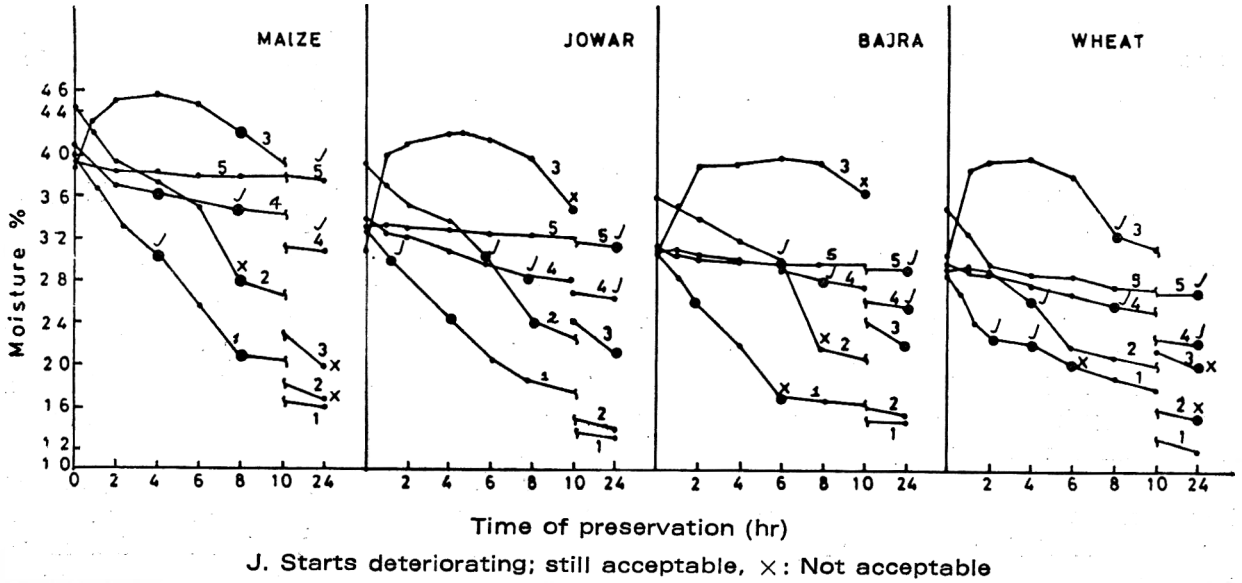

Fig. 1. Moisture content and eating quality of *rotis* preserved under different conditions

1, Open container; 2, open container after adding extra moisture 3, wet cloth; 4, closed container; and 5, polythene bag.

more time for baking the *roti* than others. Maize *roti* had a soft texture and its chewing count was less than that for the others. Wheat *rotis* were most chewy and had the highest chewing count. It was also found that the eating quality of maize *roti* was good even after keeping for 4-6 hr after baking, when compared to *rotis* from other millet or wheat flours which tended to become dry, rubbery or overchewy.

The eating quality and the moisture content of the *rotis* kept under different conditions are presented in Fig. 1. The *rotis* started deteriorating in eating quality within 2 hr of keeping in open containers in all cases except in maize which remained still soft and moist. This may be due to the fact that maize dough had more water than the others (Table 1). Though the baking time (120 sec) was more, residual moisture in the maize *roti* was still high as compared to those in wheat, jowar or bajra *rotis* immediately after baking or even after keeping for 4-6 hr in open containers. Eating quality of the *rotis* kept in closed container or polythene bags was quite good even after 24 hr although there was development of a little stale smell. Moisture content of more than 22-24 per cent at the time of tasting seems to be necessary for acceptable eating quality in the *rotis*. Among the *roti/chapati* samples, wheat *chapati* was most chewy while maize *roti* was the softest (Table 1). Addition of about 30 per cent maize or sorghum flour reduced the chewiness in the *rotis*.

Dosa: It has been reported earlier that acceptable *dosa* could be obtained from maize, sorghum and bajra by wet grinding followed by overnight fermentation[5]. However, in instant *dosa* mix formulations using the flours, difficulties were encountered in obtaining the desired texture in the *dosa*. Maize *dosa* offered more difficulty during preparation when compared to sorghum or bajra *dosa*. Maize *dosa* stuck to the pan, lacked strength and broke while turning over and had an overmoist and slimy texture while *dosa* from sorghum did not have the above defects and had good consumer acceptance. An explanation for this can be offered in terms of the paste viscosity characteristics.

Paste viscosity: At equal slurry concentration, the cold paste viscosity of batter from maize (both yellow and white) was much higher than that of the others, except for black gram flour which is known to have very high viscosity[6] (Fig. 2). The same was true of batters from *dosa* blends from the flours. The high viscosity of *dosa* batters from maize may be responsible for sticking of the maize *dosa* to the pan.

The hot paste viscosity characteristics from the amylographic studies given in Table 2 present an interesting pattern. The peak viscosity as well as the viscosity on cooling to 50°C were lower for maize than for the others indicating that on boiling or baking, maize slurry does not set to a viscous gel and has a relatively greater free flowing characteristic. This would explain the rather overmoist, slimy texture of *dosa* from maize. Reduction of water content in the batter reduced this tendency but affected spreadability and made *dosa* very thick. Longer baking at slightly lower temperature and frequent turning over of the *dosa* slightly improved the texture due to greater evaporation of water.

Vermicelli: As maize, sorghum and bajra have no gluten, dried vermicelli prepared from these grains com-

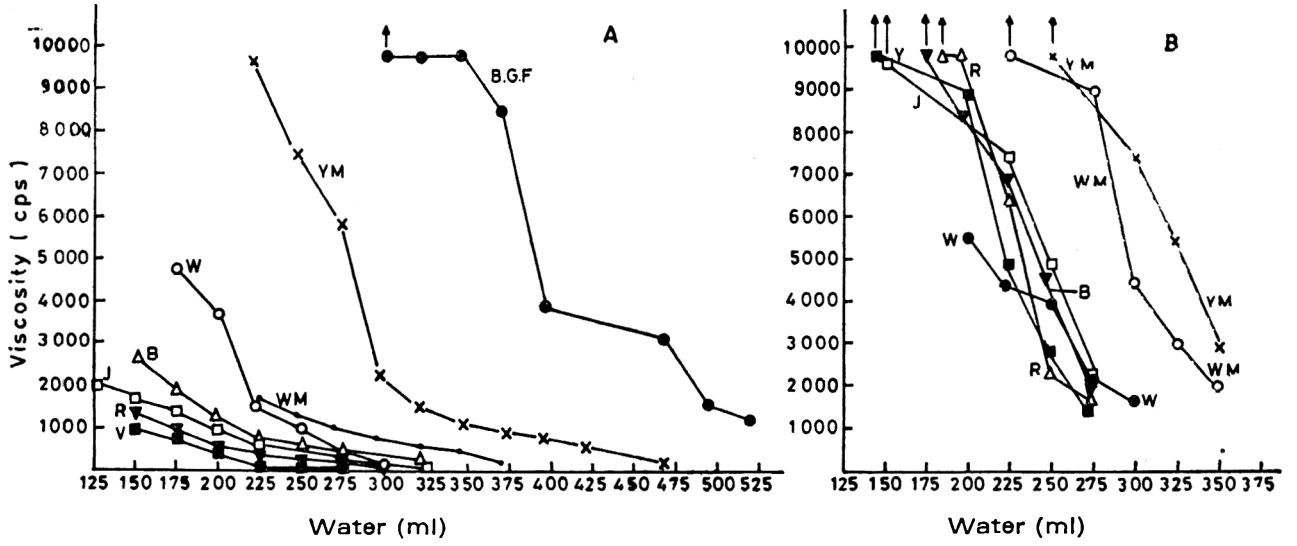

Fig. 2. Viscosity of batters of various flours dispersed in water at different dilutions at room temperature

A. Cereal flours. B. *Dosa* mixes using mixtures of black gram and the various cereal flours.

R, rice; W, wheat; WM, white maize; YM, yellow maize; B, bajra; V, varagu; BGF, black gram dhal flour.

TABLE 2. VISCO-AMYLOGRAPHIC CHARACTERISTICS OF DIFFERENT GRAIN FLOURS

Flour	Gelatinization temp. (°C)	Viscosity (BU)		
		Peak	After 20 min cooking	On cooling to 50°C
Rice	73.5	510	500	1080
Wheat	82.0	290	180	400
White maize	70.5	190	200	485
Yellow maize	73.5	140	170	360
Sorghum	73.0	260	240	565
Bajra	75.0	280	230	530
Varagu	72.0	360	310	930
Black gram	70.5	480	300	400

TABLE 3. CHARACTERISTICS OF VERMICELLI FROM MAIZE, SORGHUM AND BAJRA STEAMED FOR DIFFERENT PERIODS BEFORE DRYING

Steaming time (min)	% loss of solids and appearance of cooked vermicelli		
	Maize	Sorghum	Bajra
0	85.6*	83.8*	100.0*
10	68.2*	66.0*	100.0*
20	34.8**	28.2**	90.4*
30	21.3***	16,1***	85.0*

*Vermicelli was mashy
**50-60% shape retained
***80-90% shape retained
} based on visual examination of cooked vermicelli spread on a plate.

pletely disintegrated on cooking. Data on effect of steaming of the vermicelli before drying on its subsequent cooking characteristics are given in Table 3. Even steaming for 20 min was insufficient to give enough physical strength to enable the retention of shape during cooking. Steaming for 30 min conferred better strength and prevented disintegration during cooking to a large extent in case of maize and sorghum. Solid loss in the cooking water was however, high even in samples steamed for 30 min. Vermicelli based on bajra did not show any improvement in cooking quality. Admixture with wheat flour could be expected to improve the strength of vermicelli from the millet flours.

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Studies on Linseed *(Linum usitatissimum)* as a Protein Source for Poultry. 1. Processes of Demucilaging and Dehulling of Linseed and Evaluation of Processed Materials by Chemical Analysis and with Rats and Chicks

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A procedure for removal of mucilage from linseed with acidified water was standardised and also one for preparation of dehulled linseed. Various processed materials were analysed for proximate composition and amino acids and also subjected to nutritional evaluation with rats and chicks.

Linseed is one of the four major oilseed crops of India. Its oil finds important uses in paints and other industries. The cake is generally used as cattle feed. Linseed protein is reported to be superior to groundnut protein, but the cake finds little use in poultry feeds because of the presence of some nutritionally deleterious and toxic constituents[1-4]. Even the mucilage of linseed has been found to cause beak necrosis and other adverse effects[5].

Our research investigations were aimed at processing linseed to make it suitable as a protein source in poultry nutrition. As a preliminary, we tried various processing methods to remove mucilage and hull, standardised the methods and evaluated the products by proximate composition and amino acids, and by nutritional studies with rats and chicken. The results of these studies are presented in this communication.

Materials and Methods

Linseed was purchased in one lot to avoid batch variations, cleaned and sorted, rejecting the flat and infested seeds. The cleaned material was safely stored for various studies.

Demucilaging: Linseed samples were first analysed for mucilage content by the chemical method[6]. Then the variables likely to affect the yield of mucilage were studied as follows: Dry clean seeds in 25-g lots each were soaked in water, and in 0.25, 0.5 or 1.0 per cent aqueous solutions of commercial conc. HCl (about 36 per cent w/w; sp. gr., 1.18); seed to fluid ratio was varied as 1:2.5, 1:5 and 1:10; after presoaking for 16 hr mechanical stirring of the whole mass was done for 15 min; finally two successive washings were given with the respective fluids of original soaking. The residual mucilage on the processed seeds was judged by physical feel method, while the extracted mucilage in the pooled soaking fluid and washings was estimated by the chemical method. The selected variables, leading to maximum demucilaging of seeds, were then integrated into a standard method, described later.

Dehulling: Dehulling by hand was first done to get the relative values of hull and cotyledons present in linseed, as follows: clean seeds, in 10-g lots, were demucilaged; the moisture of the wet demucilaged seeds was adjusted with blotting paper to about 55-60 percent, which had been found optimum for easy separation of the cotyledons from hulls; the moisture adjusted seeds were decorticated by hand, and the dry weights of hulls and cotyledons separately determined. Methods such as lye-treatment used in dehulling of sesame seeds[7], and a floatation method and modified floatation method used for linseed[8,9] were also tried. In the end, based on observations in hand dehulling, a method was standardised as described later.

Preparation of defatted meals: Defatted meals of whole linseed and demucilaged linseed were prepared by expeller pressing followed by solvent extraction in food grade hexane. The dehulled cotyledon flakes were solvent extracted without prepressing. The three defatted materials were milled and designated as the linseed meal (L), the demucilaged linseed meal (L_m), and the dehulled linseed meal or the linseed protein concentrate (L_p) respectively.

Nutritional evaluation with rats: For growth studies

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with rats, the diets were prepared according to Osborn *et al*[10]. and Chapman *et al*[11]. The common ingredients of minerals, vitamins and oil constituted 13 percent of each diet. The test materials contributed the desired protein levels, and balance of the bulk was made up with corn starch.

21-day old weanling albino rats were used in growth studies, with 12 animals in each group. Feed intakes and weight gains were measured, and feed efficiency ratios (FER) and protein efficiency ratios (PER) calculated. At the end of 4-week growth period 6 rats from each group were sacrificed for carcass and liver analysis.

Nutritional evaluation with chicks: One-day old vaccinated chicks of Arbor Acres Broiler-strain were wingbanded, weighed and randomly sorted into groups of 40 each. Different groups were reared on different diets in standard brooders. In each diet per 100 parts, the common ingredients (ground corn 27.0+dicalcium phosphate 1.5+calcium carbonate 1.5.+Dl-methionine 0.1+ Coccidiostat-Bifuran supplement 0.1+vitamin mixture 4.4+mineral mixture 3.8 parts) provided the feed requirements other than bulk calories and proteins. The latter were derived in the control diet by 61.6 parts of a mixture of groundnut meal, maize and wheat in the proportion of 31.6: 14.0: 10.0 respectively. In other diets, this mixture was substituted by linseed materials to provide different levels of protein replacement as stated. Since the linseed meals had higher protein content than the mixture in the control, the balance of 61.6 was made up with corn starch to maintain isonitrogenous levels in different diets.

Feed and water were *ad lib.* The weights of individual birds in different groups and the group-wise feed intakes were taken at weekly intervals, and the mortality noted as and when it occurred over the experimental period.

Analyses: Proximate composition was determined by the AOAC methods[12]. Protein was calculated as N×6.25. Amino acids in the acid hydrolysates of different materials were calculated after resolution on the Single Column Beckman Multichrome Automatic Amino Acid Analyser at the Centre for Plant Physiological Research at Wageningen in Holland (courtesy, Mr. C. H. Vonk). Tryptophan was estimated microbiologically[13] in the alkaline hydrolysates, and the available lysine determined chemically[14].

Results and Discussion

Demucilaging and dehulling processes: Linseed cotyledons, carrying most of the oil and protein, lie inside a seedcoat which comprises a hull and outer covering of mucilage. The hull has two parts. The outer true hull is highly fibrous and tough, containing no oil and protein, while the inner soft hull contains some oil and protein. However, the two hulls are difficult to separate and, therefore, treated as one for all practical purposes. The hull and mucilage constitute about 40 per cent of the total seed. Since our chief objective was to develop linseed as a source of protein for poultry, as a preliminary, the mucilage and hull were removed successively to get two products, one merely demucilaged and the other free from even the fibrous hull.

The values of mucilage, hull and cotyledons in linseed are reported to be variable[5]. The chemically estimated 6.5 percent mucilage in our samples compared well with the literature values[6]. The chemical method is, however, very cumbersome for demcilaging large quantities of linseed. For that purpose, various conditions were studied. The results are presented in Table 1.

From these results, the following processing steps were taken as optimum for demucilaging linseed: soaking seeds for 16 hr in 1:10 w/v ratio in 1 per cent solution of commercial conc. HCl, mechanical stirring of the whole mass for 15 min and decanting the fluid; then one wash with the same dil. HCl and another with water. These results also indicated that the feel method (touch) gave a fairly accurate idea of removal of mucilage. The 16 hr period was convenient for overnight soaking. The above method was found quite satisfactory for demucilaging linseed in bulk trials.

The hand dehulling of linseed gave average yields of about 38-39 per cent hulls and 50 percent cotyledons,

TABLE 1. EFFECT OF VARIABLE CONDITIONS ON REMOVAL OF MUCILAGE OF LINSEED

	Treatments	Mucilage	
		Residual on seed	Extracted (% of seed wt.)
Water	1:2.5	++++	—
	1:5.0	+++	2.50
	1:10	+++	2.50
0.25% HCl	1:2.5	++++	—
	1:5.0	++	3.50
	1:10	++	4.50
0.5% HCl	1:2.5	++++	—
	1:5.0	++	4.50
	1:10	+	5.25
1.0% HCl	1:2.5	++++	—
	1:5.0	+	5.50
	1:10	—	6.50

Residual mucilage on seed by feel method: (++++) all water absorbed and no follow-up possible, and in others the extent of mucilage was indicated by (+++) very high, (++) high, (+) still some, and (−) almost none.

Extracted mucilage was chemically estimated and the values are average of 4 replicates.

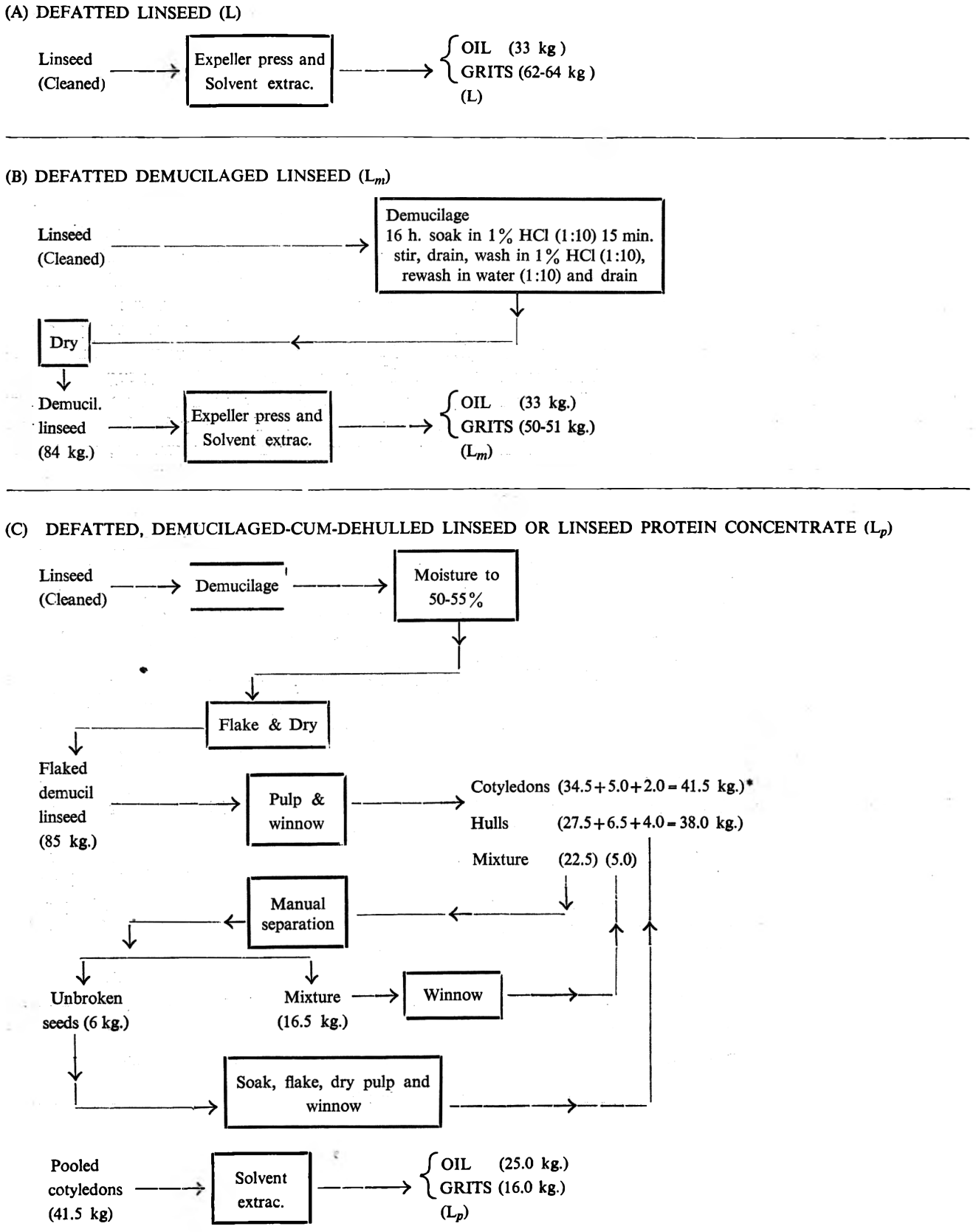

Fig. 1. Processing flow-sheet for defatted meals of (A) Linseed (B) Demucilaged linseed and (C) Demucilaged-cum-dehulled linseed or Linseed protein Concentrate (starting material in each case 100 kg.)

TABLE 2. PROXIMATE COMPOSITION OF LINSEED MATERIALS

Material	Mineral matter	Ether extract	Crude fibre	Crude* protein
Linseed meal (L)	5.2	8.4	10.1	36.6
Demucilaged linseed meal (L_m)	5.0	8.6	11.8	43.4

All values as percentage on moisture-free basis.
*N×6.25

with the mucilage and moisture removed during processing and drying. These values were comparable with others reported in the literature. Hot alkali solution, found suitable for sesame dehulling,[7] failed in linseed even at as high as 10 percent concentration. The reason for this may be the greater thickness, and higher proportion, of a tougher seed coat in linseed than in sesamum. In sesamum, the seed coat forms only about 15 percent of the total seed. The floatation methods[8,9] also failed. We did not pursue these approaches further, since the experience of hand dehulling gave us lead to a simple technique involving the following steps: The demucilaged seeds, after moisture adjustment to 55-60 percent, were passed through a flaking machine to get 0.25-0.3 mm thick flakes; the flaked material was dried in a cross-flow oven; and then passed through an APV pulping machine; the final separation of hull and cotyledons was done by winnowing. Large scale trials gave yields of about 41 parts cotyledons, 38 parts hull and 5 parts inseparable cotyledon-hull mixture from 100 parts of starting material as shown in Fig. 1.

Proximate composition: The proximate analysis of two linseed materials L and L_m, is presented in Table 2. The third material, L_p, was analysed only for N and had 55.6 per cent crude protein. The values for linseed meal (L) were within the range of other studies[15]. As effect of processing, following generalisations are possible. Removal of the non-fibrous and non-nitrogenous mucilage led to some increases both in crude fibre and crude protein in the demucilaged meal (L_m). Removal of hull led to further increases in the crude protein content.

Amino acid analyses: Tryptophane in alkali hydrolysates of various materials was found to be around 1.5 g per 16 g N. The spectrum of other amino acids is presented in Table 3. In general, the pattern of unprocessed linseed meal (L) in our studies was similar to that reported by other workers[16-18]. In common with other plant proteins, there is the usual deficiency of S-amino acids, but adequacy of all others. From the preliminary analyses presented here, the effects of processing on the amino acid patterns of different linseed materials may be summarised as follows:

Between the unprocessed (L) and the two processed (L_m and L_p) linseed materials, there was not much difference. If anything, the demucilaging suggested some improvements, particularly with reference to a few essential amino acids. This may be due to, on one hand, mucilage interference in estimations, and on the other, removal of some good quality protein with the hull.

The available lysine values for different materials were in the range of 2.31-2.62 g/16 g N. As part of the total lysine in respective materials, the value of available lysine were in the 58-65 per cent range. In general, the available lysine followed the relative pattern of total lysine values, broadly suggesting no serious effects of processing.

Rat growth studies: Table 4 presents the results of 4-week growth studies with rats on different diets. The control diet had 10 percent protein from casein, and the other three from linseed (L), demucilaged linseed (L_m) and the dehulled linseed (L_p) meals respectively, with L_p also in two more diets at 15 and 20 percent protein levels respectively.

All diets at 10 percent protein level, including casein control, showed similar feed intakes. When the diets had higher linseed material to give 15 and 20 percent protein levels there was an even increased feed intake.

Growth on all three linseed diets at 10 per cent protein level was lower than on casein diet. Among themselves, the L_p diet gave a lower growth response than the

TABLE 3. AMINO ACID (g/16 gN) ANALYSES OF ACID HYDROLYSATES OF VARIOUS DEFATTED LINSEED MATERIALS*

Material**	Threonine	Isoleucine	Leucine	Lysine	Methionine	Phenyl-alanine	Tyrosine	Valine	Arginine	Histidine	Glycine
L	4.38	3.61	5.22	4.03	1.37	4.55	2.77	4.30	10.66	2.37	6.2
L_m	4.57	4.04	6.03	4.48	1.53	4.53	2.32	4.79	11.13	2.38	6.14
L_p	4.03	3.91	5.74	3.78	1.78	4.60	2.16	4.66	10.39	2.16	5.80

Courtesy: C. R. Vonk, Centre for Plant Physiological Research, Wageningen, Holland
**L–Linseed meal; L_m–demucilaged linseed meal; L_p–dehulled linseed meal or linseed protein concentrate.

TABLE 4. RAT GROWTH EXPERIMENT: 21 DAY OLD WEANLING ALBINO RATS WERE KEPT ON DIFFERENT DIETS FOR FOUR WEEKS FOR GROWTH RESPONSE AND CONVERSION EFFICIENCY RATIOS

Group	Diets*	Av. food intake (g)	Gain in wt. (g) Mean±S.E.		F. E. R. Mean±S.E.		P. E. R. Mean±S.E.	
A.	Linseed meal (L_{10})	153.41	39.70		0.259		2.47	
B.	Demucilaged linseed meal ($L_{m\,10}$)	157.80	41.01		0.261		2.50	
C.	Dehulled linseed meal ($L_{p\,10}$)	156.94	30.85	±3.15	0.197	±0.116	1.87	±0.10
D.	Dehulled linseed meal ($L_{p\,15}$)	173.44	53.60	(45 df)	0.310	(45 df)	2.32	(45 df)
E.	Dehulled linseed meal ($L_{p\,20}$)	202.52	84.20		0.415		2.14	
F.	$Casein_{10}$	154.75	52.35		0.338		3.28	

*Subscribed numerals indicate protein levels from the source material.
S.E.: Standard error of the mean; d.f.: degree of freedom
Results of test of significance by Duncan's Multiple Range test at 5% level.

Diet	C	A	B	F	D	E
Av. gain in wt. (in 4 weeks)	30.85	39.70	41.10	52.35	53.60	84.20
Average FER	0.197	0.257	0.261	0.310	0.338	0.410
Diet	C	E	D	A	B	F
Average PER	1.87	2.14	2.32	2.47	2.50	3.28

Note: (*a*) Any two means not underscored by the same line are significantly different.
(*b*) Any two means underscored by the same line are not significantly different.

L and L_m diets. At 15 percent protein level, however, the L_p diet showed a growth response comparable with casein diet, and at 20 percent protein level even better.

Feed efficiency ratio (FER) on different diets showed a trend similar to the growth response. PER on the diets at 10 per cent protein levels also showed a similar trend. But the PERs on diets with L_p at 15 and 20 percent protein levels were progressively lower, in contrast to the growth and FER trends.

From the rat growth study, one may generalise as follows. The incorporation of linseed materials into diets had no adverse effect on their palatability for rats, irrespective of the presence of mucilage and hull. Nutritionally, as reflected in growth responses and the feed and protein efficiency ratios, the linseed materials were inferior to casein, and among themselves the linseed protein concentrate (L_p) most inferior. Evidently, removal of mucilage had no beneficial effect in rat nutrition, but dehulling led to some quality deterioration, probably from removal of some protein fraction and/or certain growth promoting factor/s. A better growth response and a higher FER at higher protein levels from the dehulled linseed protein concentrate (L_p) suggest the linseed protein to be of medium nutritive value. Such vegetable proteins promote good growth at higher levels[19], when their amino acid make up is not very inferior[20,21,22]. The absence of a significant difference between casein at 10 percent protein and the L_p at 15 percent suggests the availability of amino acids, quality-wise, in comparable amounts[23].

Though growth response and PER are common parameters of evaluation of protein quality of feeds, they have serious drawbacks. The increase in weight may also be due to fat accumulation. Therefore, the carcass and liver of the rats of different groups in this experiment were also analysed.

Carcass and liver analysis: The moisture content of the carcass fell between 66.4 and 72.4 per cent, and of the liver between 69.5 and 73.9 per cent, suggesting no difference due to feed quality variations. The fresh weight of liver, ranging between 3.93-4.16 g. per 100 g body weight, also reflected the same thing. The N analyses are given in Table 5. The final weights of rats after 4-week experiment represent the growth trend on

TABLE 5. RAT GROWTH EXPERIMENT. NITROGEN ANALYSIS OF CAR CASS AND LIVER OF RATS AFTER THE 4-WEEK GROWTH EXPERIMENT

Diets*	Final wt. (g)	Carcass (m Ng/g d.m.)	Liver (m Ng/g d.m.)	Carcass-N (% of N intake)
L_{10}	79.3	65.6	80.7	69.0
L_{m10}	83.6	64.5	78.1	60.1
L_{p10}	66.2	66.9	79.3	53.3
L_{p15}	91.0	64.3	81.2	50.4
L_{p20}	124.5	69.1	90.6	40.6
$Casein_{10}$	94.7	69.2	87.3	85.7

*See table 4 for details of diet symbols

different diets. The carcass N and the liver-N on dry matter basis showed no significant differences between different diets. The carcass-N as per cent of N-intake showed linseed materials to be inferior to casein, and among themselves the unprocessed linseed meal (L) better than demucilaged linseed (L_m), and the latter superior to the linseed protein concentrate (L_p), with the last one showing even lower values at higher protein levels in the diets.

Good retention of N in the carcass is a measure of protein quality[24]. Some adequacy of linseed in this direction has already been suggested[25]. We have further evidence that the mucilage has no adverse role in rat nutrition. A lower N retention in case of dehulled linseed diets (L_p) indicates a positive role even of the hulls. A beneficial role of whole linseed meal has also been suggested in earlier studies[26].

Chick experiments: At 100 per cent protein replacement by linseed meal (L_{100}), almost 90 per cent and at 75 per cent replacement (L_{75}) about 70 percent chicks died within the first week of feeding. At 50 percent replacement (L_{50} and $L_{m\,50}$) about 40 per cent deaths occurred in the first week, and another 20 per cent in the second week. Only at 25 per cent protein replacement (L_{25} and $L_{m\,25}$) the mortality was low in the early stages of feeding. Table 6 shows the final results of a 6-week experiment with chicks on a control diet and on the other diets containing linseed (L) and demucilaged linseed meals (L_m) at different protein replacement levels. As the deaths began within the first few days of the feeding, it was not possible to determine growth in relation to feed intake. However, the feed intake was generally low, as also the average weight of the birds surviving at the end of 6 weeks, compared with the control group chicks which showed good growth and no mortality.

The mortality in chicks, soon after feeding suggests that the linseed meals contain certain constituents, highly toxic to chickens. The presence of several toxicants in linseed have been reported[1-4]. We found that even demucilaging led to no improvement in chick nutrition.

Summary and conclusions: Processes were standardised for step-wise removal of mucilage and hull from linseed. Proximate composition analyses indicated the following: removal of the non-nitrogenous mucilage and of the low-nitrogen hull, led to progressive increases in the crude protein content of the demucilaged linseed (L_m) and the linseed protein concentrate (L_p) respectively from amino acid analyses, one may generalise that there was no effect of processing, even on the availability of lysine.

Nutritional evaluation of linseed meals with rats showed them to be of medium protein quality and indicated no advantage of removal of mucilage and hull for rat nutrition. But the chick experiments showed the presence of high toxicity in the linseed meals, even after the removal of mucilage. From our experiments, we can state that the linseed toxicity to chicks could not be demonstrated in rat experiments, probably due to species differences. For chick nutrition, more work is needed to develop a suitable process of detoxification of linseed.

TABLE 6. MORTALITY AND WEIGHT OF SURVIVING CHICKS FED LINSEED MEAL WITH DIFFERENT PROTEIN REPLACEMENT LEVELS

Diet*	Mortality** (%)	Final av wt of surviving birds (g)
Control	—	690
L_{25}	7.1	302
L_{50}	58.5	179
L_{75}	83.3	—
L_{100}	90.0	—
L_{m25}	7.1	272
L_{m50}	61.9	193

One day old chicks in groups of 40 each were fed a control standard diet and other diets containing linseed (L) and demucilaged linseed (L_m) at different protein replacement levels for 6 weeks.

*L—Linseed meal, L_m—demucilaged linseed meal, with the subscribed figures indicating the protein replacement levels.

**Mortality only due to feed effect, excluding the accidental deaths.

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ERRATA

The following corrections are indicated in the article "Detection of Ambadi (*Hibiscus cannabinus*) seed oil in vegetable oils" by M. R. Grover and T. V. Mathew published in this Journal 1978, **15**(4), 154-155.

In page 154, Fig. 1, under legend "condensed oil" should be read as "cotton seed oil".

In page 154-155: the last line of 154 should be connected to the second line in page 155 followed by first line when it will read as the UM of Ambadi seed oil was characterised by a specific prominent blue spot at R_f. 0.15, easily recognisable even at 5% concentration from other faint spots various colours (including bluish hue) at about this R_f shown by other oils.

LIPID COMPOSITION OF PEARL MILLET FLOUR

The content of free and bound lipid in two hybrids and five varieties of pearl millet in (g/100 g) flour varied from 2.85 to 4.37 and 0.37 to 0.02 respectively. Nonpolar and polar fraction of free lipid constituted in the range of 2.65 to 4.10 and 0.17 to 0.31 respectively. Nonpolar fraction in the bound lipid, was in the range of 0.09 to 0.16 whereas the polar fraction was in the range of 0.23 to 0.29. The ratio between nonpolar and polar fraction of total lipid is high which makes it less suitable for breadmaking.

Recently, there has been considerable interest in the improvement of pearl millet (*Pennisetum typhoideum*). A break through in production was achieved by the exploitation of male sterile lines and introduction of high yielding hybrids[1]. The lipid content of pearl millet[2] flour is very close to that of maize[3] and is considerably higher than other cereals[4]. Daniels and co-workers[5] have suggested that a study of lipid distribution could lead to better understanding of dough mixing and baking. Limited information on lipid characterization and fractionation for millets is available[6,7,14]. This work was therefore, undertaken to evaluate quantitatively various fractions of pearl millet lipid from hybrids and varieties of Indian origin.

Seeds of five varieties and two hybrids grown under uniform agro-climatic conditions were ground to pass through 40 mesh sieve. Free lipids were extracted using diethyl ether[8] and extracted with methanol: chloroform: water (2:10:0.8) for bound lipids[9].

Protein was removed from the bound lipids by making the extract solvent free and traces of water eliminated by adding anhydrous sodium sulfate and finally repeatedly extracting the lipid with chloroform. The chloroform extracts were pooled, dried, and weighed for bound lipids. The polar and nonpolar lipids were determined by partition method[10]. Total free lipid was separated on silica gel G column into four fractions; hydrocarbon, sterol esters (I), containing triglycerides (II), free fatty acids, sterols, partial glycerides (III), and phospholipid (IV) using serial elution with 80 ml n–hexane (I), 200 ml benzene (II), 150 ml diethyl ether (III), and 100 ml methanol (IV)[11]. Sterol[12] and free fatty acids[13] contents were estimated in fraction III; partial glycerides were calculated by subtracting the content of sterol and free fatty acids from fraction III.

The lipid composition of pearl millet flour is presented in Table 1. Total lipid content ranged from 3.4 to 4.78, of which free lipid was 2.8 to 4.48 and bound lipid 0.37 to 0.42 g/100g flour. Tevekelev[14] reported 3.95 per cent lipid content in millet.

The nonpolar fraction of the free lipid ranged from 2.65 to 4.1 per cent and polar fraction from 0.17 to 0.31 per cent. In case of bound lipid the nonpolar fraction ranged from 0.23 to 0.29 per cent. The ratio of nonpolar to polar fraction ranged from 11.9 to 20.15, 0.25 to 0.67 and 5.93 to 7.84 for free, bound and total lipids respectively. Variety 'S-350-49' with a maximum ratio of 20.15 for nonpolar to polar in free lipid shows for bound lipid a minimum ratio 0.35. On the other hand, variety 'T-55' with a ratio of 11.19 for nonpolar to polar in free lipid has the ratio for bound lipid increased to 0.56. In other words, in the varieties and hybrids in which nonpolar to polar ratio is low in bound lipids, the ratio is high in free lipids. This indicates that these varieties and hybrids tested have almost equal potential for the synthesis of polar and nonpolar lipids and it is the distribution of free and bound lipids which differs in the varieties and hybrids.

TABLE 1. LIPID COMPOSITION OF PEARL MILLET FLOUR OF DIFFERENT VARIETIES

Variety	Free lipid (g/100 g flour)			Bound lipid (g/100 g flour)			Total lipid (g/100 g flour)			
	Nonpolar (N)	Polar (P)	N/P ratio	Nonpolar (N)	Polar (P)	N/P ratio	Nonpolar (N)	Polar (P)	Total	N/P ratio
TF23A×Bil 3-B	2.65	0.20	13.25	0.14	0.28	0.50	2.80	0.48	3.28	5.93
TF23A×Bil 3-A	3.15	0.22	14.12	0.13	0.29	0.45	3.28	0.51	3.79	6.24
T-55	3.22	0.29	11.19	0.15	0.27	0.56	3.38	0.56	3.94	6.04
302/2/C	3.82	0.31	12.33	0.14	0.23	0.60	3.96	0.54	4.50	7.27
Georgia 519-71	4.10	0.27	15.24	0.11	0.26	0.41	4.21	0.53	4.74	7.84
S-350-49	3.39	0.17	20.15	0.09	0.27	0.35	3.48	0.44	3.92	7.84
R-47-1-2-2	3.92	0.30	13.23	0.16	0.24	0.67	4.07	0.53	4.60	7.37

TABLE 2. CONSTITUENT PRACTIONS OF FREELIPIDS OF DIFFERENT VARIETIES (MG PER 100 MG OF FREE LIPID)

Variety	Hydrocarbon & sterol esters	Triglycerides	Free fatty acids	Sterol	Partial glycerides	Phospholipids
TF 23 A×Bill 3-B	1.65	64.20	21.99	0.87	3.14	8.15
TF 23 A×Bill 3-A	6.20	70.36	11.37	0.34	1.78	9.95
T-55	2.31	73.08	13.05	0.52	5.64	5.40
302/2/c	8.88	60.72	18.45	0.62	4.93	6.40
Georgia 519-71	1.94	65.21	22.97	0.68	5.03	5.17
S-350-49	3.5	70.86	14.20	0.62	3.68	7.10
R-47-1-2-2	2.12	74.56	10.24	0.61	5.77	6.70

The nonpolar content is more as compared to polar in free lipids whereas in the bound lipids polar content is higher than the nonpolar. But overall in the total lipids (free and bound lipids) the nonpolar component predominates. Pomeranz *et al*[15]. have demonstrated that polar lipids improve the loaf volume of bread. The ratio of nonpolar to polar lipids in pearl millet flour was in the range of 5.93-7.84 and that of wheat flour was 1.13 to 1.20 as reported by Stevan and Houstan[4] and calculated from the results of Pomeranz *et al*[16]. In this way, millets have much higher nonpolar to polar ratio as compared to wheat flour lipids. The nonpolar content of the total lipids is higher as compared to polar and this high nonpolar content makes it less suitable for breeding. Among these varieties and hybrids, the ratio of nonpolar varies in a narrow range.

Further study of various fractions of free lipids (Table 2) showed that hydrocarbon and sterol esters were 1.65 to 8.8, triglycerides 1.7 to 5.7 and phospholipids 5.4 to 9.9 per cent.

Critical evaluation of the data revealed that the varieties 'T-55' and 'R–47–1–2–3' have comparatively higher amount of triglycerides and less amount of free fatty acids. The result indicates a possible interconversion of triglycerides and free fatty acids. Hydrocarbon and sterol esters fraction showed a wide range of variation with respect to hybrids and varieties. Unlike other fraction, this fraction was found to be heterogenous by thin layer chromatography. Major portion of the nonpolar pigments was noted to have come in this fraction. Phospholipids estimated by the column chromatography were found to be quite comparable to this amount obtained by solvent partitition method[10].

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DETECTION OF FOOD COLOURS BY GEL ELECTROPHORESIS

This new method of detecting water soluble colours from foods consists of extraction of colours from foods by water and alcohol (80%), followed by running the colour in polyacrylamide gel using citrate buffer (pH 2.4) as chamber buffer. This method is rapid and takes only 1 hr and can detect very minute amount of dyes (10 ppm). The greatest advantage of this method is the by-passing of wool dyeing which is often used for clean up in similar process of paper or thin layer chromatography. Indigocarmine and few other blue-green dyes are affected specially by alkali in wool dyeing.

Polyacrylamide gel electrophoresis which is not much used for separation of small molecules has recently been applied by Dong Bor Yeh[1] for the separation of water soluble colours. However, this method has not been found very suitable in resolution of colours. In this communication, an improved method of application of polyacrylamide gel electrophoresis in the separation and detection of water soluble synthetic colours from foods is described.

Preparation of polyacrylamide gel: The gels (7.5 per cent polyacrylamide) were prepared as follows: Solution A was prepared by taking 24 ml of N HCl, 18.15 g tris-base and 0.23 ml N,N,N^1,-N^1,-tetramethyl ethylenediamine in 100 ml flask and making up the volume to 100 ml with water.

Solution B was prepared by taking 30.0 g of acrylamide and 0.8 g bisacrylamide in a flask and making up the volume to 100 ml. Solution A (4 ml) and Solution B (3 ml) were added to 5 ml of ammonium peroxodisulphate, $(NH_4)_2S_2O_8$), (12 mg solid dissolved in 5 ml distilled water).

The above solution mixture is poured into gel tubes (7×0.5 cm). The bottom ends of the tubes are closed by parafilm secured with a rubber band.

Water (3 mm) is poured carefully over the acrylamide layer. The tubes are exposed to a fluorescent light for about 15 min to complete polymerisation.

Chamber buffer—Citrate buffer (pH 2.4) is prepared, by mixing 10 ml M citric acid, with 1.51 ml M NaOH, and making up the volume to 100 ml. The pH was checked.

Extraction of colours from different foods is done as follows:

Spices: Two grams of the sample is shaken with 20 ml water for 10 min., filtered and filtrate concentrated to 1 ml.

Milk: To 10 ml of milk is added 40 ml 95 per cent alcohol and allowed to stand for 30 min. It is then filtered and filtrate concentrated to 1 ml.

Other food products: Ten gram of the sample is shaken with 40 ml of 80 per cent alcohol containing one drop of ammonia (in case of green and blue coloured compounds ammonia was not added) for 30 min. Filter and concentrate the filtrate to 1 ml.

Electrophoresis was carried out for 45 min using a current 10 mA per gel. The sample size was 20-50 μl and to each sample 10-20 per cent of sucrose was added.

Eleven dyes, permitted in India[2], as such and as mixtures added to different foodstuffs in varying amounts were detected by this method. This method can detect upto 100 ppm dye in a sample.

Blue FCF and Fast Green FCF give two bands each as also found by Yeh[1], and Green S gives three bands. The multiple bands were observed here even with apparently pure samples, obtained from appropriate bands from preparative TLC and with dyes of single TLC band, kept over night in contact with the gel; hence either subsidiary dyes are not separated in the so called pure sample or the dyes undergo change during electrophoresis. Dilution of chamber buffer has no effect on the resolution of dyes. The mobility of the dyes decreased slightly with the increase of the volume of the sample. This method gives better resolution of dyes yielding discrete bands in comparison to Yeh's method, where an alkaline buffer was used instead of an acidic buffer. This probably is critical as preponderance of ions-for full ionisation in alkali—of the spices being separated is probably less suitable than their slow release in acid. Further, the gel in alkaline becomes sticky and cannot be easily removed from the tubes. In several experiments clean-up of the dyes by wool-dyeing has not been found to be necessary. This is an advantage as it eliminates the possibility of destruction of blue-green dyes specially Indigo Carmine. Natural colours do not interfere in this method, and for this reason spices can be analysed easily. In wool-dyeing of spices there is always an interference by natural colours and other components. It may be possible to quantitatively estimate the colours by using densitometry[3]. This method is also applicable to drugs for detecting dyes present in them.

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PESTICIDAL ACTION AND STABILITY OF PHOSALONE IN DRY FISH

Phosalone, an organophosphorous insecticide has been found suitable for use at the rate of 70 ppm in preventing infestation in dry fish by *Lasioderma serricorne* (Fabricius). A slightly higher dose of 85 ppm is necessary if the fish is already infested. It is also found that the pesticide is stable during storage, but it can be removed almost completely by washing and cooking. The phosalone preserved dry fish has got acceptable appearance.

Phosalone 0, 0-diethyl S-(6-chloro-2-oxobenzoxolin-3-yl) methyl) phosphorodithioate, an insecticide and acaricide has the property of a broad spectrum pesticidal activity. It is active on all important mites and many insects. Its use is recommended in many countries for the treatment of various crops, i.e., grapes, rapeseed, sugar beet, potato, alfalfa, tea, tobacco, etc[1]. The toxicity studies in rats and dogs have confirmed its low toxicity. Its acceptable daily intake for man has been arrived at 0.006 mg/kg body weight[2].

In the present communication the use of Phosalone in controlling insect infestation in dry fish is reported. Infestation of dry fish is a problem in many places.

The insects occurring in the stored dry fish (Bombay duck) was identified as *Lasioderma serricorne* (Fabricius), Family: *Anobiidae*, Order: *Coleoptersa.*

Pieces of dry fish (Bombay duck) each weighing about 3.5 g and of nearly identical size (5×2.5×0.75 cm approximately) were sprayed separately, with 70, 140 and 280 ppm of Phosalone uniformly on the surface of the fish. The untreated fish served as control. The treated and untreated fish were taken separately in conical flasks (1 l. capacity). Five insects (*L. serricorne*) per piece were introduced into each conical flask. The mouth of the flask was covered in such a way that the air circulation remained normal and stored in the sheif at ambient temperature (25-40°C) and relative humidity (80-95 per cent) as it was carried out in June-August.

Phosalone residue on the treated samples was measured by TLC (unpublished work) during storage and after washing and cooking (Table 3). Washing was done using hot water. Cooking was done by first frying in oil (200°C) with spices and condiments for 5 min followed by boiling in water for 20 min. Estimation is done on the whole curry.

TABLE 1. ACTION OF PHOSALONE ON DRY FISH

Phosalone level (ppm)	Time required for complete kill of insects after application
70	3 days
140	1 day
280	12 hr
Nil	All alive

The results of the effect of Phosalone are given Table 1.

The residual amounts of the Phosalone on the pieces of fish were found not altered even at the end of three months after application as listed in Table 2.

TABLE 2. PHOSALONE RESIDUE IN THE TREATED DRY FISH

Time interval (days)	Residue level (%)
7	100
14	100
21	100
45	100
60	100
90	98

However, considerable lowering of residue level occurred during washing and cooking. It will be seen from Table 3 that the combined effect of washing and cooking completely removed the pesticide in the dry fish. It can be concluded that Phosalone can be applied at the rate of 70 ppm to protect the dry fish from the attack of *Lasioderma serricorne;* lower doses (not reported herein) are not very effective. It was also observed that a slightly higher dose of 85 ppm of Phosalone was necessary to kill the insects already present in dry fish.

TABLE 3. LOSS OF PHOSALONE DURING WASHING AND COOKING

Operation	Residues recovered (%)
Washings	40
Washed fish	50
Washed and cooked fish	Negligible

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BOOK REVIEWS

Developments in Food Analysis Techniques—1: Edited by R. D. King Applied Science Publishers Ltd., London 1978 pp. 323, price 44 $

This book outlines the principles of modern methods in food analysis giving references to original sources. The subject matter is contributed by eleven specialists.

Chapter one covers methods for the estimation of vitamins in foods, and problems encountered during analysis and reporting of results. Chapter two deals with determination of nitrogen by the classical Kjeldahl and Dumas procedures and the newer methods viz. neutron and proton activation analysis. Protein estimations include those based on chemical and physical methods. Chapter three deals with the role of water in foodstuffs and includes thermodynamics of equilibrium phenomena, methods for the determination of equilibrium relative humidity and bound water, their scope and limitations. Chapters four and five deal with the applications of high pressure liquid chromatography and gas chromatography respectively, in food analysis viz. analysis of flavour components, food additives, pesticides, mycotoxins etc. Enzymic methods for the detection and assay of a variety of food components have become feasible with the availability of highly purified enzymes, the newest in the field being those based on immobilized enzymes, which are covered in chapter six. Chapter seven highlights the use of the ion selective electrodes in the determination of specific ions in foods. Automated methods in food analysis, dealt in chapter eight, indicate their usefulness and include methods followed in the Laboratory of Government Chemist, London, for specific foods and contaminants. Chapter nine on the determination of carbohydrates in foods is restricted to estimation of free sugars. Clarifying agents used prior to analysis and estimation of sugars by different methods are discussed. The last chapter on the use of atomic absorption spectroscopy in food analysis gives a brief description of the instrument and methodology for the determination of a number of trace metals.

The get-up of the book is good and it contains a number of useful tables, illustrations and the subject index. Extensive references are given under each chapter.

The book is not merely a review of methods but a compendium of critical commentary on modern methods in food analysis. It is a useful reference book for food analysis and research workers who could look for details from the references cited.

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Food Processing and Nutrition: by A. E. Bender, Food Science and Technology Monograph, Academic Press, 1978 pp. 243. price $ 15.25, £ 7.80.

Food technology has made rapid strides in standardising new processes/techniques for development of food products of high consumer appeal, texture and packaging. But the effect of processing on nutritional aspects of food attracted wide attention only lately. As such available literature in this field is neither extensive nor conclusive. With increasing consumer awareness of nutrition, research in this area is growing at an accelerated pace and the need of a well documented book on the subject is imperative.

The book under review presents in a concise form the existing data in a very lucid manner.

The book is divided into five sections. Section A comprises of two chapters describing the general principles governing the importance of loss of nutrients during processing in the first chapter and the beneficial effects of processing in the second, which includes the detoxication of legumes, destruction of trypsin inhibitor in cereals and the liberation of bound niacin.

Section B entitled 'Effects on Nutrients' gives the effect of processing on vitamins, proteins, carbohydrates, lipids, mineral salts and the stability of nutrients. The effect on individual vitamins has been discussed exhaustively so as to cover all aspects of loss of vitamins during processing.

Changes in proteins, during processing have been enlisted in Chapter 4, with the protein terminology as an introduction. Various changes such as denaturation, Maillard reaction, changes in lysine and cystine have been given with appropriate illustrations.

Section C reviews the effect of various processes such as pasteurisation, sterilization, blanching, canning, fermentation and other heat processes on nutrients.

The fourth Section D presents the effect of processes on all food commodities such as meat and meat products, milk, cereals, fruits and vegetables, pulses, oil seeds etc. The effect on the two important com-

modities, meat and meat products and cereals has been dealt with extensively with reference to all nutrients and processes.

The last Section E deals with enrichment of nutrients and regulations governing enrichment.

The book contains more than 900 references thus being a very exhaustive collection of literature in the field. It fulfills the long felt need of the student and researcher and should find a place in all leading libraries concerned with food science research/teaching.

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Food Legume Processing and Utilization with Special Emphasis on Application in Developing Countries. prepared by Alvin Siegel and Brian Fawcett and published by International Development Research Centre, Box 8500, Ottawa, Canada K1G 3H9, 1976, pp. 88.

The book entitled "Food Legume Processing and Utilization with Special emphasis on application in Developing Countries" prepared by Alvin Siegel and Brian Fawcett and published by International Development Research Centre is yet another attempt by international agencies to stress the importance of food legumes in enhancing the quantity and quality of proteins in the diets of people in developing countries where intake of animal foods is low and the diets are predominently starchy in character. The present book describes the traditional methods of processing and utilization and suggests how modern technology can help in improving the traditional methods both in the processing and utilization. The review is prefaced by an account of the antiquity of legume grains in historic and prehistoric times and current trends in production and world trade.

The main chapter on the traditional processing and utilisation covers the primary and elementary processing for seed coat removal to produce *dhal* or split legumes, methods of soft cooking of the beans or legumes by boiling in water, and other methods of processing of legumes by roasting, toasting or high temperature puffing, and preparation of deep fried products. Sprouting or germination of legumes for enhancing nutritive value and improving consumer acceptability and the chemical changes that take place during this vital process have also been dealt with. The preparation of autofermented foods or fermented foods inoculated with pure microbial cultures for preparation of *Idli*, *Dosa*, *Miso*, *Natto*, *Tempeh*, etc. has been described at length. The practice of agglomeration which is somewhat similar to the process of granulation practised in making sago is an interesting practice prevalent in Africa. Among the traditional processes which have been practised on a commercial scale, milling into *dhal* and canning of beans and other legumes to produce ready-to-eat products have been described at length. The nutritonal implications of the canning process have also been described.

The second main Chapter is devoted to the description of the application of improved technologies for processing legumes. These include the improved process for *dhal* milling developed in India and Canada using improved equipment for moisture reduction and for decortication. The newer work on producing quick cooking legumes by appropriate hydration, cooking, and drying has been described at length. The precooked legume powder whose hydration property can be controlled by appropriate processing technology has great possibility for application, as the product can serve as a convenient ready-to-use product by urban groups even in developing countries. Protein rich isolates from legumes by dry method (air classification) or by wet method (precipitation) and their relative use fulness have been adequately described in detail. The review ends with consideration of utilisation of by products of the legume processing industry for feed and industrial uses.

The treatise gives useful data on world production, yield and trade. The review is useful for specialists in nutriton, food processing and also those interested in enhancing the protein status of adults and children in developing countries.

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The Care and Management of Farm Animals: Ed by W. N. Scott: Bailliere Tindall, London 2nd Ed, 1978. pp. 254. Price £ 7.95.

The book entitled "The care and management of farm animals" is a practical textbook which deals with various systems of production and husbandry of farm animals. It contains up-to-date and comprehensive information on farming of livestock which is so essential for veterinary and animal science students.

The first edition of the book was published as "The UFAW Handbook on care and management of Farm Animals" by Churchill Livingstone, during the year

1971; in response to the interest of the readers in the area of welfare of farm animals. The present edition is a useful informative text for all those who are interested in the field of animal welfare, veterinary and agriculture science. The book contains fifteen chapters covering farming of dairy cattle, beef cattle and veal calves, water buffalo, sheep, goat, swine, rabbit, horse, mules donkeys, milk, farm dogs and poultry with notes on camel and elephant. Besides, it also deals with humane killing of animals in the farm, metabolizable energy requirements and legislation in United Kingdom concerning farm animal welfare.

The editor deserves all praise for the excellent efforts he has made in amalgamating older useful information available on animal farming with the new systems that have developed in recent years. Information contained in the book on camels and elephants is scanty and these sections need enlargement in future.

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Trees, Food and People: Land Management in the Tropics, by J. G. Bene, H. W. Beall and A. Cote, IDRC, Box 8,500 Ottawa, Canada KIG, 3 Hg, 1977, pp. 52, Price $ 1.

The importance of tropical forests to mankind and the alarming pace of destruction or conversion of these tropical forests into unproductive waste lands ultimately leading to the formation of deserts has been very well stressed. The diversity of the tropical forests, their over exploitation, under use and environmental significance, cannot be over emphasised. The composition of the tropical forests has been well brought out in the book. It has been predicted that within next 25 to 30 years most of the humid tropical forests will be transformed into unproductive waste land and the deterioration of Savanna into deserts will continue at an ever increasing speed. This statement is very true to the syllabi considering the pace of over exploitation of the forests in different tropical countries to day.

The burning problem is whether the natural forests could be converted into man made forests of fast growing species at the cost of changes in eclogical balances, which of course would not be prudent if adopted all over. Rehabilitation of the worked tropical forests with canopy lifting and tending after intensive utilisation of the bio-mass and other economic products would be the best manner of conservation of these tropical forests. Where forests are over exploited and cannot be retrived they should be better converted to man made forests of mixture of species adopting multiple forestry practices.

It is very apt expression that wisely managed semi arid areas will grow two or three times more biomass than unmanaged land produces. The tropical forests yield not only timber but many other economic forest products, besides being the abode.

Trees for People—An account of the Forestry Research Programme supported by the International Research Centre: *IDRC*, Box 8500, Ottawa Canada, KIG 3Hg, 1977, pp. Prize $ 1.

The planting of shelter belts—fuel plantations, village woodlots, forage trees etc., is an age old practice. It is only recently due to population explosion and increase in the animal population in all the developing countries, there has been an adverse effect on the existing vegetation. The intensive demand for fuel, fodder and fertilizer has been staring us in the face. Valuable cowdung is burnt as fuel for want of alternative fuel. The countrysides are parched and dessicated by the hot sun. The only manner in which the problem can be solved is by a massive programme of social forestry under which the raising of shelter belts, windbelts, village wood lots are all included. Agro-forestry is thus an important instrument of land transformation especially where we are dealing with problems like shifting cultivation in many tropical countries.

The large scale implementation of these methods though purported to be the best manner of land use have to be demonstrated to the people by experimental and demonstration plots. The IDRC should be congratulated on the launching of Research Programme in the several allied problems and it is sure to have a great impact on the adoption of proper land use methods. The book is interesting and is an asset for reference in any research organisation.

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Guide to Hygiene and Sanitation in Aviation: by J. Bailey, World Health Organisation, Geneva, Rome 1977, pp. 162; Price 28 Sw. fr.

This booklet is a well written and comprehensive one, covering all aspects of the various areas that come under the purview of Hygiene and Sanitation in today's world

of civil aviation with its high speed jet aircraft and rapid movement of large masses of passengers.

This reviewer would like to make it clear that a thorough, detailed reveiw of all the areas covered is not really possible, and would like to point out that each area is in itself a specialist one. This reveiw is therefore, of necessity confined to general remarks.

3. *General Considerations*

These admirably outline the basic principles that should be considered important.

4. *Food*

All the recommendations outlined under this heading are sound and thorough, and if put into practise would give us high standards of Hygiene in the aviation catering world. However, in the reviewer's personal experience such high standards are only paid lip service This is of course particularly so where commercial catering firms are used, due to the profit motive Stringent inspection procedures to be followed by penalties should be enforced. Again, particularly in the Gulf areas, S. E. Asia, and the Far East, monopolists generally hold the catering concessions, and effective control over quality control, hygiene standards etc. is not available due to the political background. Even in the West, the trade unions make it practically impossible for a slovenly worker paying no attention to the demands of his work in the interests of hygiene to be removed from his job.

Again, the Guide recommends specialised catering equipment etc., these may be easily available in the West, but in the majority of the 3rd. World Airports and Airlines (where the standards of hygiene and sanitation most need upgrading) budget allocations go for items that are more visible on the surface, and indeed, the most suitable equipment may not be at all available. The Guide would have done well to include recommendations for simpler equipment and procedures, which, whilst they may not have the same effect, would still produce adequate measures for improving present day standards in many locations. It should also be recommended that special sealed containers for storage of ice for drinks be availed of, too often the ice is supplied in polythene packets which are stored in large, open bucket type containers. During the flight this issued by passengers as a depository for trash.

6. *Toilet Sanitation*

It should recommend that a separate toilet be set aside for the cabin crew in all aircraft, for their exclusive use. Cabin crew duties will not allow them to stand patiently in line amongst passengers so that they may wash their hands as often as they collect air sickness containers, clear up spillage of food and drinks both in the passenger cabin and in the galleys, after helping mothers with infants, and prior to meal and beverage services. This latter practise is most important due to the high incidence of stomach trouble amongst crews, due to various reasons; some innocent, and some definitely potentially dangerous on the grounds of hygiene.

It should also be strongly recommended that aircraft toilets are situated in a completely different zone from food storage areas and galleys.

8. *Aircraft Interior Cleaning*

This section gives a good list of cleaning schedules, but once again in practise these are more ignored than observed.

In conclusion, it may be mentioned that Inspection procedures that are recommended are all very well, but unless carried out by rotating Inspectors with no warning, and then backed up by stern penalties where necessary, mere inspection is not enough. If the Inspectors findings were made public, perhaps the public's reaction to airlines airports, and caterers that were found to be lax would ensure a high standard of voluntary hyigene and sanitation procedures!

Mr. Bailey was formerly the Hyigene Officer with British Airways, and responsible for much of the Hygiene education given to employees of BA. As such, he may have included a recommendation for mandatory Hygiene and Sanitation procedures to be taught to all Airport cleaning staff, airline cleaning personnel, and catering services employees, as well as to cabin crew. In the majority of cases, lapses in high standards are caused more by genuine ignorance than anything else.

S. R. VALLURI
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Shri C. P. Natarajan Appointed as Acting Director of CFTRI

Shri C. P. Natarajan, Deputy Director, Central Food Technological Research Institute (CFTRI), Mysore, and the Past President of AFST assumed charge as acting Director of the institute with effect from 1 November 1978, consequent on the Director Dr. B. L. Amla's taking up a three-year assignment as Senior Food Technologist at the World Bank, Washington.

Shri Natarajan (born 8 Sep. 1921) obtained his B.Sc. (1941) and M.Sc. in chemistry (1943) from the Madras and Andhra Universities respectively. After working as Assistant Biochemist in the Indian Institute of Science, Bangalore, in a Madras Government scheme during 1943–45, he proceeded to USA under a Government of India scholarship and obtained M.S. in food technology from the University of California, Berkeley, in 1947. He was an honorary travelling fellow in food technology at the California University in 1946–47 and was elected to Sigma XI and the Institute of Food Technologists, USA.

Joining CFTRI in September 1950 as Junior Scientific Officer, Shri Natarajan rose to the position of Scientist E and chairman, Discipline of Plantation Products & Flavour Technology in 1964, and Scientist F in 1972.

Shri Natarajan has carried out extensive research in chemistry and processing of planatation products. His major contributions include: systematic data generation on the chemical composition of coffee grown in different regions of the country (the data have served as the basis for framing the specifications by the Indian Standards Institution and the Central Committee for Food Standards); solution of problems faced in storage of coffee beans; development of indigenous know-how for making soluble coffee; development of processes for preparing instant tea from green tea leaves, oleoresins and essential oils from spices, dehydration of green pepper, retention of green colour of cardamom to cater to the market demands in countries in West Asia and complete technology for an indigenous flavour concentrate for manufacture of a soft drink, being presently marketed in India under the brand name '77'.

Shri Natarajan is responsible for establishing a school of coffee and tea research at CFTRI. Many practical applications in the area of roasting, brewing, packaging and storage of coffee and detection of adulteration of coffee have emerged from researches carried out under his guidance. He and his research team, working on spices, won the Rafi Ahmed Kidwai Award (1977) for their work on major spices.

Shri Natarajan has taught for over 14 years post-graduate students in food technology, and, during 1971–72 was in-charge of the International Food Technology Training Centre at CFTRI.

Shri Natarajan was contracted for a period of four months during 1975–76 by the Industrial Development Centre for Arab States for preparing a blue-print and programme for the establishment of the Institute of Food Industries in Sudan. He was the member/leader of the Indian delegation to the meetings of ISO on spices/tea, held in Sri Lanka in April-May 1978. He visited Bulgaria and USSR as exchange scientist in 1972 and 1973. His visit to West Germany in 1975 resulted in a programme of cooperation between India and that country in R & D in food technology under a CSIR-KFT agreement.

In addition to being associated with several ISI committees, Shri Natarajan, is a member of Indian Spices Development Council and Indian Cashew Development Council and their research and development cells; and a member of Coffee Quality Committee of the Indian Coffee Board. He is a member of the ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS (INDIA) and was the President of the Association in 1977. He is also a member of the Society of Biological Chemists, India; Indian Chemical Society; Association of Microbiologists of India and New York Academy of Sciences.

Shri C. P. Natarajan has authored or co-authored more than 200 research papers.

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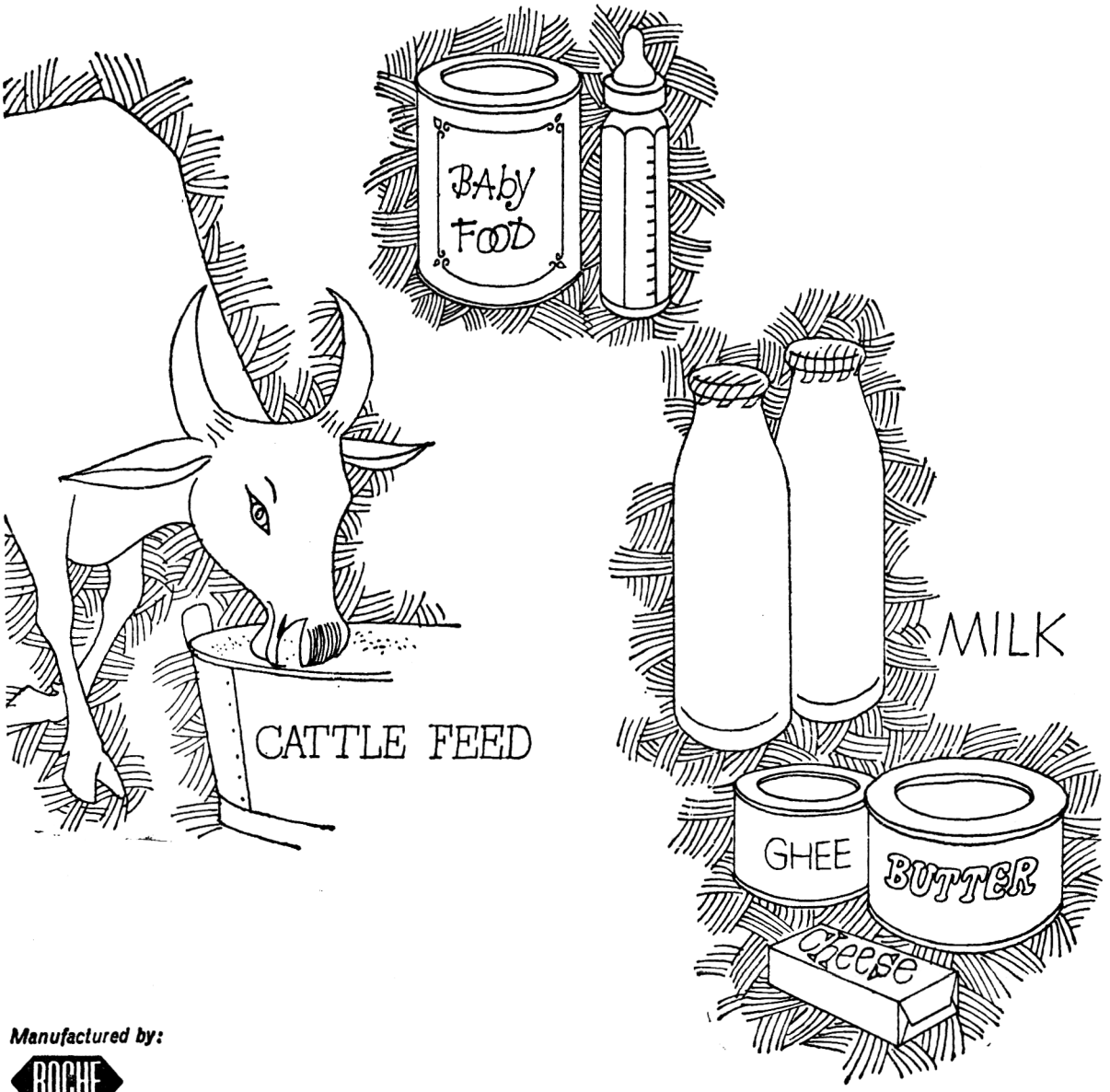

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1. Manuscripts of papers should be typewritten in double space on one side of the paper only. They should be submitted in **triplicate.** The manuscripts should be complete and in final form, since no alterations or additions are allowed at the proof stage. The paper submitted should not have been published or communicated anywhere.

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

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

4. **Abstract:** The abstract should indicate the scope of the work and the principal findings of the paper. It should not normally exceed 200 words. It should be in such a form that abstracting periodicals can readily use it.

5. **Tables:** Graphs as well as tables, 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. Nil results should be indicated and distinguished clearly from absence of data.

6. **Illustrations:** Line drawings should be made with *Indian ink* on white drawing paper preferably art paper. The lettering should be in pencil. For satisfactory reproduction, graphs and line drawings should be at least twice the printed size. Photographs must be on glossy paper and contrasty; *two copies* should be sent.

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

   (*d*) *Proceedings, Conferences and Symposia:* As in (*c*).

   (*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.

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