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JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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Role of Hydrolytic Enzymes in the Spoilage ot Fish

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The involvement of hydrolytic enzymes in the autolytic spoilage of fishery products has been investigated in two varieties of fish. The major source of hydrolytic activity in marine fish, Bombay duck (*Harpodon nehereus*) was contained essentially in the drip, while in fresh water fish, *Tilapia mossambica* it was in the skin. The accumulation of hydrolytic end products like inorganic phosphate, E^{260} absorbing compounds and tyrosine during progressive autolysis of the muscle tissue could be correlated with the release of cathepsin D activity. The deskinning of the fillets of *Tilapia mossambica* and the removal of drip from Bombay duck muscle provide suitable methods for suppressing enzyme mediated spoilage in these two varieties of fish.

The action of hydrolytic enzymes associated with the lysosomes of skeletal muscle constitutes one of the important factors responsible for the deterioration of stored fishery products[1]. Besides modifying the quality attributes such as texture[2] and flavour[3], these enzymes are known to promote growth of bacteria by providing essential nutrients in the form of low molecular weight tissue degradation products[4]. Despite several investigations on the role of catheptic enzymes on meat tenderisation[2,5], information on the autolytic breakdown of fish tissue constituents mediated by acid hydrolases is largely speculative. The present paper reports on the distribution of lysosomal hydrolases in the skeletal muscle and skin of fresh water fish, *Tilapia mossambica* and in the muscle and drip of marine fish, Bombay duck (*Harpodon nehereus*). The influence of these enzymes in causing spoilage processes in fish was ascertained by measuring the liberated hydrolytic end products during the progressive autolysis. The data on the effect of removing skin and drip from *Tilapia mossambica* and Bombay duck respectively on the enhancement of shelf stability are also included.

Materials and Methods

Tilapia mossambica of 14-16 cm average length, acclimatized for two weeks in fresh water in a laboratory tank, were killed by decapitation. All the operations were carried out at 0-4°C unless otherwise stated. After evisceration, deboning and deskinning, the skeletal muscle was finely minced with the scissors. For storage experiments, fish fillets with or without skin were prepared separately. Freshly caught Bombay duck were brought to laboratory in iced condition. Skeletal muscle samples were prepared as described above. The drip from Bombay duck was collected by holding a batch of fish at -20°C for 3 days and thawing it at 5°C subsequently.

Preparation of homogenate: The minced fish skeletal muscle samples were homogenized in 0.25 M sucrose containing 0.2 M KCl for 2 min employing Sorvall Omnimixer at a rheostat adjustment set at '3'. The homogenates of Bombay duck and *Tilapia* were made up to 10 per cent (W/V) and the pH adjusted to 7.2. The homogenates were filtered through two layers of cheese cloth.

The skin homogenate was prepared in sucrose-KCl medium by grinding with sea sand.

Enzyme assays: The activities of acid phosphatase (EC 3.1.3.2), acid ribonuclease (EC 2.7.7.16) and cathepsin D (EC 3.4.23.5) were determined from muscle and skin homogenates and drip by using the methods described by Barrett[6]. Protein content was estimated by the procedure of Miller[7].

Short term autolysis in muscle homogenate: Since the marine fish (Bombay duck) spoils faster than the fresh water fish (*Tilapia mossambica*), the skeletal muscle of this fish was taken up for short term autolysis experiment to ascertain enzyme mediated spoilage. Portions of Bombay duck homogenates were taken in previously sterilized Erlenmeyer flasks and treated with a drop of toluene and stoppered. The homogenates were incubated at 37°C in a shaker water bath, for 0-6 hr. At regular intervals the autolysis was terminated by adding either 10 per cent trichloroacetic acid or 10 per cent perchloric acid containing 0.25 per cent uranyl acetate. After cooling, the samples were centrifuged and the levels of hydrolytic end products liberated were determined from appropriately diluted aliquots.

Autolysis of Bombay duck drip was also conducted similarly.

Estimation of hydrolytic end products: Amino acids and peptides expressed as tyrosine equivalents and inorganic phosphate were measured from the 10 per cent TCA supernatant according to the method of Miller[7] and Fiske and Subbarow[8] respectively. Compounds possessing absorbancy at 260 nm were measured spectrophotometrically in the PCA supernatant and are expressed as equivalent of oligonucleotides (adenylic acid).

Evaluation of quality and autolytic degradations during prolonged storage: Skin-on and deskinned *Tilapia* fillets were packed separately in radiation sterilised polycell pouches and stored at 0-4°C. At regular intervals, the samples were evaluated for appearance, odour and texture by a 5–member panel. Organoleptic scoring (OS) was done based on a 9–point hedonic scale[9]. A portion of both skin-on and deskinned samples was used for preparing homogenate in sucrose-KCl medium. The activity of cathepsin D[9], the levels of accumulated hydrolytic end products and total volatile basic nitrogen (TVBN) value[10] were measured from the homogenates.

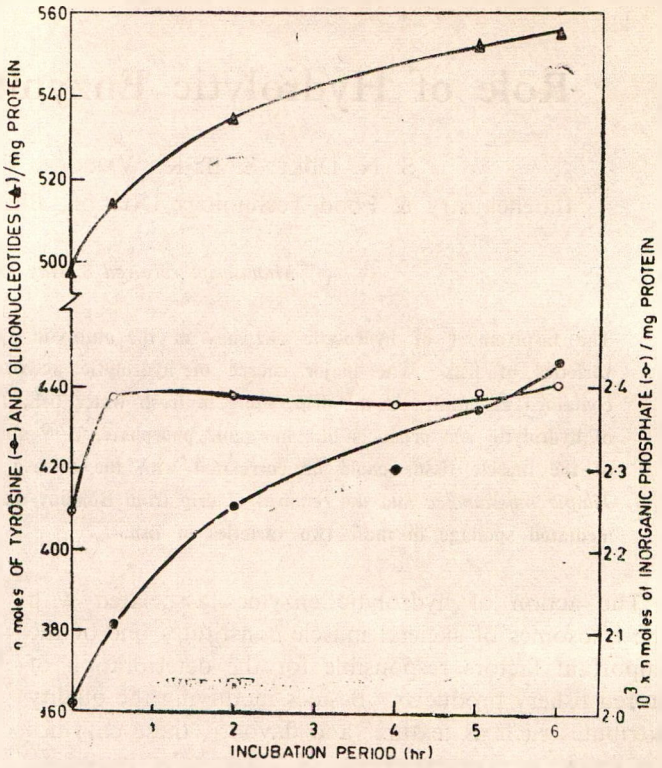

Fig. 1. Pattern of accumulation of end products during the autolysis of Bombay duck drip at 37°C.

Results and Discussion

The distribution profiles of lysosomal enzymes in the skeletal muscle and drip of marine fish Bombay duck as well as in the muscle and skin of fresh water fish *Tilapia mossambica* are incorporated in Table 1. Although these represent total specific activities, lysosomal nature of these enzymes is apparent from properties, such as acidic pH dependence and structure linked latency[11,12]. However, in the drip and skin, only total activity could be measured, since the enzymes remain completely in the soluble (non-bound) form. A comparison of enzyme activities in muscle with that of drip or skin clearly shows that these two form rich sources of acid hydrolases, containing nearly 10 times higher activity than the muscle.

Table 1. PROFILE OF LYSOSOMAL HYDROLASES IN FRESH WATER FISH, *Tilapia mossambica* AND MARINE FISH, BOMBAY DUCK (*Harpodon nehereus*)

Fish	Cathepsin D	Acid RNase	Acid PO_4 ase
Bombay duck			
Muscle	3.20	8.00	9.60
Drip	59.16	296.72	75.76
Tilapia mossambica			
Muscle	34.13	44.00	79.00
Skin	319.00	606.80	312.00

The activity of the enzyme represents total specific activity and is expressed as nmoles of products liberated/mg protein/hr.

Owing to low enzyme content, the rate of liberation of end products in skeletal muscle of Bombay duck was apparently slow. The results presented in Fig. 1 indicate the pattern of autolysis in Bombay duck drip during incubation at 37°C. The rapid spoilage of Bombay duck during storage has been attributed to extensive exudation of drip resulting from the denaturation of structural proteins and loss in water holding capacity of the muscle[13]. Our data indicate that the lysosomal hydrolases present in the drip also mediate in the fish spoilage by extensively degrading the tissue constitutents. Although accumulation of phosphate remained steady following the first 30 min of incubation, products of proteolysis and E^{260} absorbing compounds maintained a progressive increase in their liberation during the entire period of incubation indicating that cathepsin D and acid ribonuclease could be the major enzymes involved in the elaboration of autolytic events. In view of this, along with hydrolytic end products, the release of cathepsin D was also measured from skin-on and deskinned *Tilapia* fillets during prolonged storage at 0-4°C as shown in Fig. 2 and 3. Deskinning the fish resulted in the decrease in cathespin D activity as well as reduction in the formation of amino acids and pepti-

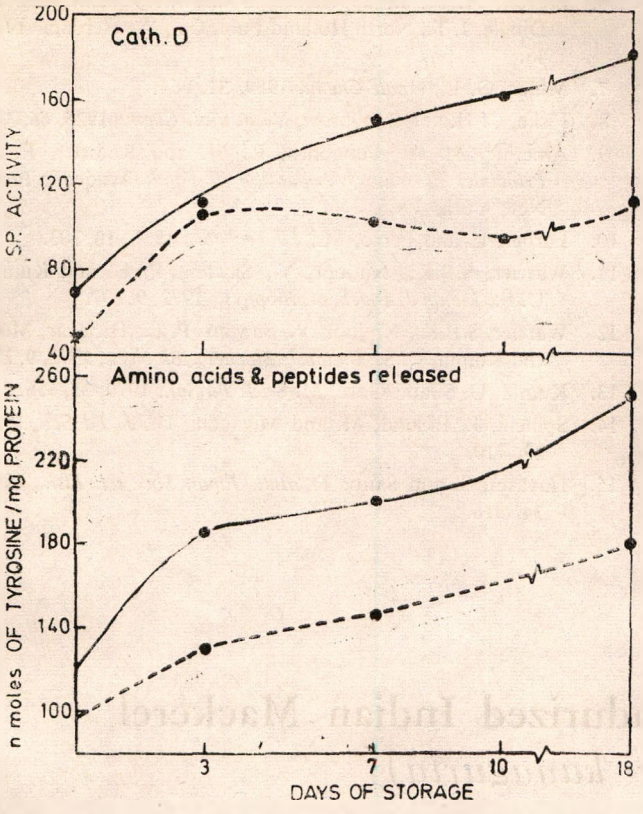

Fig. 2. Autolytic changes in skin-on and deskinned *Tilapia* fillets stored at 0-4°C.
———, Skin-on; ....., Deskinned.

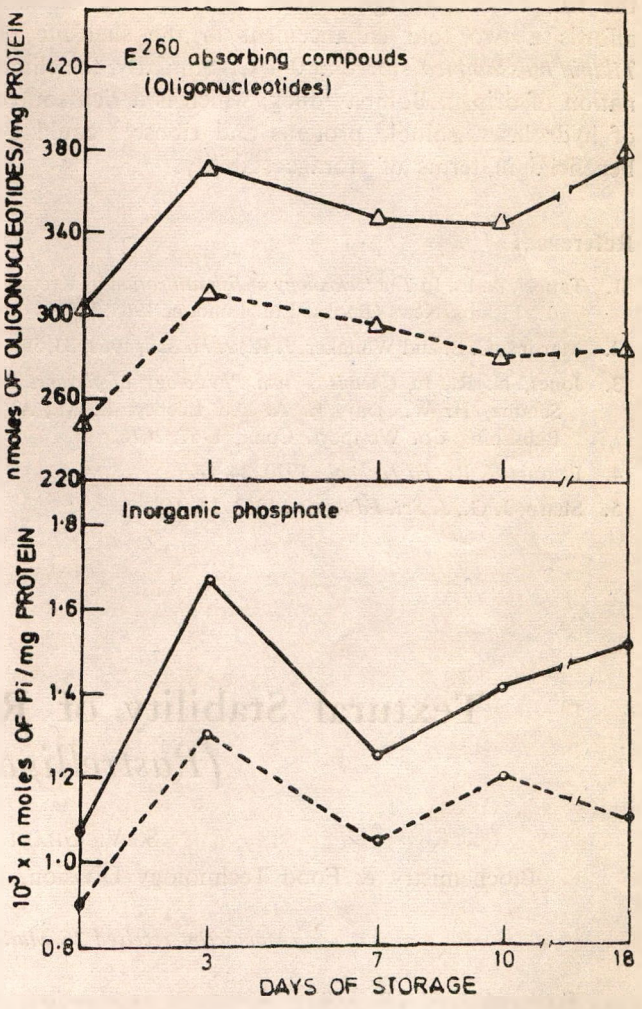

Fig. 3. Autolytic changes in skin-on and deskinned *Tilapia* fillets stored at 0-4°C.
———, Skin-on; ....., Deskinned.

des. A decline in the accumulation of phosphates and nucleotides, was also noted in the stored, deskinned, samples. When the stored fish fillets were evaluated for acceptability, it was observed that skin-on samples spoiled on the 10th day, while deskinned ones were acceptable even on 18th day of storage. The data on the organoleptic evaluation and assessment of TVBN level included in Table 2, thus clearly point to the advantages of deskinning the fish for extending the shelflife. The action of tissue proteinases in general and cathepsin D in particular has been attributed to the undesirable textural changes in stored fishery products[4].

TABLE 2. ORGANOLEPTIC SCORE[1] AND TVBN[2] VALUES OF SKIN-ON AND DESKINNED FISH FILLETS DURING STORAGE AT 0-4°C.

Storage period (days)	Type of fish fillets: Skin-on Org. score	Skin-on TVBN	Deskinned Org. score	Deskinned TVBN
0	9	22.41±3.82	9	18.48±1.30
5	8	22.41±2.32	8	21.00±2.90
10	6	24.36±1.90	8	24.76±2.41
15	2	25.20±3.53	7	28.00±2.35
20	0	43.20±3.69	6	26.32±2.63

[1] The judges in the panel for O.S. consisted of 5 members

[2] The values are expressed as mg nitrogen/100 g fish. The data represent the average of 3 experiments±SD.

The influence of enzymic degradation products of fish tissue constituents on the quality attributes has been studied by several investigators[1,4]. Products of proteolysis such as amino acids and peptides have been shown to contribute towards the overall flavour changes in stored fishery products[3]. Further, the extent of nucleotide degradation and eventual formation of hypoxanthine have been accepted as useful criteria in evaluating post-mortem quality of fish[14]. The skin of fish, apart from being a rich source of lysosomal hydrolases as shown in the present study, contains a variety of nucleotides and their derivatives especially hypoxanthine as demonstrated by Hayashi and Saito[15]. These findings also seem to emphasize the desirability of deskinning

the fish before storage. Thus, the removal of skin affords a two fold enhancement in the shelf-life of *Tilapia mossambica* stored at 0-4 °C. Similarly, the elimination of drip in Bombay duck, which is a rich source of hydrolases, soluble proteins and ribose[13] could be beneficial in terms of storage stability.

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Textural Stability of Radurized Indian Mackerel *(Rastrelliger kanagurta)*

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Fresh mackerel (*Rastrelliger kanagurta*) were subjected to 150 Krad gamma irradiation and were stored at 0-2°C and evaluated at regular intervals for changes in shear force (SF), water holding capacity (WHC) and plasticity index (PI). The SF of both control and radurized samples decreased during storage; but the radurized samples took a longer period for the same decrease in SF value. The WHC reduced and the pH increased in both control and experimental samples and the drip loss was also similar in these samples. Sensory ratings indicate that irradiated samples were acceptable for 20 days whereas control samples were acceptable only for 10 days.

Our earlier studies[1] have indicated that a radurization dose of 150 Krad could significantly enhance the refrigerated storage of Indian mackerel (*Rastrelliger kanagurta*). In these studies, quality assessment of radurised mackerel was made on the basis of organoleptic attributes, bacteriological and biochemical indices of freshness[2-4]. The use of gamma radiation for extending the refrigerated and the ambient shelflife of seafoods has been essentially based on the elimination of certain microorganisms responsible for spoilage[5-7]. However, there is paucity of information on radiation-induced changes in the textural stability of sea-foods. This paper relates to radiation-induced alterations in the textural attributes of mackerel stored at 0-2 °C, in terms of shear force (SF), water-holding-capacity (WHC), plasticity index (PI), drip loss and pH.

Materials and Methods

Processing: Fresh mackerel (*Rastrelliger kanagurta*) purchased from the local market were brought in ice to the laboratory. Whole fish were beheaded, eviscerated, washed and drained, and finally placed between folds of blotting paper to remove excess moisture. Dressed mackerel were packed in 1 kg quantities in polyethylene bags (300 gauge) which were surrounded with ice and subjected to a dose of 150 Krad gamma radiation in a Co^{60} Package Irradiator. Both radurized and unirradiated samples were stored at 0-2 °C, and at regular intervals, representative samples were drawn for evaluation.

For evaluation of physical parameters, whole mackerel were filleted and pieces from the dorsal body muscle, close to the front dorsal fin were used for shear force

(SF) measurements. The pieces were cut to standard measurements of 6-7 mm length × 3-4 mm width × 1-1.5 mm thickness. The remaining fillets were ground to paste and used for the water holding capacity (WHC) and plasticity index (PI) determinations. The average of twelve determinations were taken for each parameter.

Shear force (SF) was determined using a modification of the method described by Awad and Diehl[8]. An Instron Universal texturometer was equipped with a shear strength cell composed of five blades, each 2 mm thick and spaced 2mm part. These blades passed through a sample holding box with a corresponding number of slots, and the sample was laid acorss the slots in the box. The samples were sheared with a 6 kg force at right angles to the major surface of the sample across the fibres, at a rate of 1.6 mm/sec. The shear force (kg) was monitored by an electric recorder operating at a speed of 10 cm/min.

The method used for determining water holding capacity (WHC), was a modification of the procedure of Grau and Hamm[9]. Ground fish muscle (300 mg) was weighed on a 7 cm Whatman No. 1 filter paper which was preconditioned by keeping overnight in a desiccator over saturated KCl solution. The muscle sample kept on filter paper at room temperature was placed between two metal plates and immediately pressed under 60 kg pressure for 2 min using the Instron. The total wetted area and the meat film area were measured using a planimeter. The percentage of free water and WHC were calculated according to the formula of Awad and Diehl[8] viz: per cent free water

$$= \frac{(\text{Total wetted area} - \text{meat film area})\ 10.465}{\text{mg sample} \times \text{per cent moisture}} \times 100$$

WHC = 100 − per cent free water

The plasticity index (PI) was determined using the method of Roberts *et al*[10]. and expressed in cm^2/g. Drip loss was measured as described by Gore and Kumta[11]. The slurry prepared from 10 g muscle homogenized in 20 ml distilled water was kept aside for 15 min at room temperature before measuring pH.

Results and Discussion

Changes in SF, WHC, PI, drip loss and pH of muscle of radurized and unirradiated mackerel during storage at 0-2°C are shown in Figure I.

The shear force values in both the unirradiated and radurized samples were found to decrease during storage. The initial shear force of 2.6 kg in the control samples reduced to 1.3 kg after 10 days storage while a similar effect was noticed in the radurized mackerel only after 20 days. The WHC values also reduced

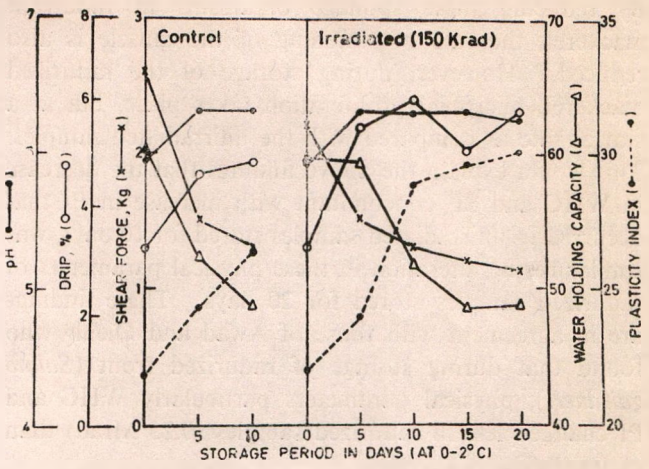

Fig. 1. Textural alterations in mackerel during storage at 0–2°C.

rapidly both in the control and radurized samples within 5 and 10 days respectively and thereafter showed a gradual decline during the remaining storage period. The drip loss was similar in both control and radurized samples in the range of 3-5 per cent. The pH of the muscle in both the control and radurized samples increased from 5.9 to 6.3 within the first 4 days and thereafter remained constant.

These results indicate that at any particular time during storage at 0-2°C, the changes in SF, WHC, PI, pH and drip loss were more pronounced in control than in radurized samples indicating that control samples spoil faster than radurized samples.

It is now fairly well established that the softening of seafoods during storage, which is manifested by SF decrease and PI increase, is mainly caused by exogenous bacterial enzymes rather than endogenous tissue enzymes[12] and that only enzymes produced by specific bacteria are mainly responsible for spoilage of fish. The present results indicate that softening of mackerel muscle during storage at 0-2°C correlates well with decrease in WHC and SF as well as with increase in PI. Sensory ratings indicated that unirradiated samples were acceptable for 10 days as compared with radurized samples which were acceptable up to 20 days of storage at 0-2°C. Again, it was found that the amount of reduction in WHC and SF, and amount of increase in PI shown by unirradiated samples within 10 days, closely resembles the changes in these physical parameters in radurized samples within 20 days. This indicates that WHC, SF and PI are good indices of spoilage of mackerel. The changes in pH and drip loss did not correlate with textural alterations during spoilage. This suggests that these two parameters are not good indices of spoilage. Radurization doses in the range of 0.1-0.25 Mrad are known to inactivate the gram-ve spoilage organisms which are comparatively sensitive to radiation.

By reducing these spoilage organisms in radurized mackerel, the rate of softening of the muscle is also reduced. However, during storage of the radurized mackerel, microbial proliferation takes place, but at a slower rate as compared with the unirradiated samples. This would explain the above findings that the decrease in WHC and SF concomitant with increase in PI that occurred in unirradiated samples stored for 10 days, was similar to the alteration in these physical parameters of radurized samples stored for 20 days. These findings are in agreement with those of Awad and Diehl[8] who found that during storage of radurized trout (*Salmo gairdneri*), physical parameters particularly WHC and PI changed less in radurized samples (0.25 Mrad) than in unirradiated samples.

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The Microbial Flora of Ready-to-cook Pork Products—A Public Health Point of View

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In the present study 95 samples of different ready-to-cook Pork products, namely Cocktail sausages, Oxford sausages, porkies, ham, bacon, and kababs were analysed for their bacterial content. A total of 388 isolates from these products were obtained. Among these were potential pathogens, usually responsible for food poisoning. The presence of such organisms highlights the public health hazard in the ready-to-cook products, this work emphasizes the urgent need for a rigorous bacteriological control of the food products in India.

There are no published data on the effect of precooking on the survival of potential bacterial pathogens in beef and pork products prepared in India. The Indian Standards Institute has also not clearly specified the bacteriological standards for such ready-to-cook products. The common occurrence of different species of bacteria in such products raises a question as to their significance in organoleptic spoilage or public health. The organisms mostly found in such products originate either as endogenous flora of the raw meats and other materials used or are added during processing operations to the products as extraneous contaminants. Therefore, microbiological 'guidelines' or 'standards' for ready-to-cook meat food products not only ensure the improvement in quality of the products but also affords protection of consumers from health hazards.

The purpose of the present study was to determine the bacterial load, the various groups of pathogenic and spoilage organisms present in the ready-to-cook pork products, sold in the city of Bombay and thereby to evaluate their hygienic standards from public health point of view.

Materials and Methods

Samples of ready-to-cook pork products were procured from the local factories before packaging for sale, by the Marketing Officers, Government of India, in Bombay city. The samples were collected in sterile (irradiated under ultraviolet light for 15 min) polythene bags, sealed by flame and were brought in ice to laboratory for analysis. The samples consisted of 29 cocktail sausages, 16 Oxford sausages, 16 Porkies (including

pork and breakfast sausages), 8 hams (cured), 10 bacons (cured) and 16 Kababs (mutton and pork).

Fifty grammes of each sample was surface washed with 100 ml of sterile Normal Saline Solution (NSS), homogenised and suspended in 450 ml of NSS. Tenfold dilutions upto 10^{-7} of the sample were made in NSS, and the last three dilutions were used for obtaining total viable counts by pour-plate method (plate count agar-oxoid) using 0.1 ml inoculum. Triplicate sets of plates were prepared from each of the dilutions for incubation at 37°C, from temperatures (30°C±1.5°C) and at refrigeration temperature (8°C to 10°C). For total viable count, the average of the three plates at different temperatures was considered for calculations. Similar procedure was adopted for surface washings of the samples. Differential counts for faecal coliforms, *E. coli*, *Staph. aureus*, *B. cereus*, *Cl. perfringens* were carried out on selective and differential media[1,2]. Identification of the bacterial species was done as per the recommended methods[3,4].

0.1 ml of 10^{-4} dilution was spread on Carter's medium[5] for spot identification of pathogenic *Staph. aureus*. Other *Staphylococcal* strains, were identified by picking out and processing the typical colonies from the blood agar spread plates with same amount of inoculum and dilution. Similarly *Bacillus* and *Micrococcus* species were enumerated and identified from blood agar spread plates[3,4]. The Egg-Yolk Agar[1] medium was used for confirmation of *B. cereus*.

Faecal *Streptococci* were enumerated on Slanetz and Bartley's Enterococcus Agar[1], and confirmed by using Mitis Salivarius Agar[5], and other standard media[4].

The number of *Lactobacilli* was determined by use of Tomato Juice Agar-Special (B-389)[2].

Enumeration of *Clostridium perfringens* was carried out using Iron Sulphite Agar[1] in high agar tubes. The strains for identification were inoculated in Robertson's Cooked Meat Medium[4], and later confirmed by standard procedures[3].

The Violet Red Bile Agar[1] and Violet Red Bile Nitrate Agar[1] were used for rapid confirmation and enumeration of the *E. coli*, and thermotolerant *Coliforms* respectively. Quantitative and rapid determination and differentiation between lactose and non-lactose fermenters was done by the use of Brilliant green Lactose Saccharose Phenol Red (BLSF) agar[2]. One ml of the 10^{-4} dilution was shown in 10 ml Selenite broth tubes and 0.1 ml from these was again plated on the BLSF agar after 24 hr incubation. The typical non-lactose fermenting colonies were inoculated in urea medium, Triple Sugar Iron agar (TSI) and Tryptone for Indol test. The Imvic tests were performed for all the gram negative isolates encountered. *Salmonella* was confirmed by use of slide agglutination test with polyvalent sera.

Serratia and *Pseudomonads* were enumerated from the plain agar pour plates and confirmed by Standard tests[3,4]. The King's medium 'A'[4] was used for enhancing the pigment produced by *Serratia*, and King's media 'A' and 'B'[4] were used for differentiation between *Ps. aeruginosa* and *Ps. fluorescens*. The determination of the psychrophilic bacteria mostly among the genera *Pseudomonas* and *Achromobacter* was carried out on Milk Peptone agar[4] and identification till species level was based on the standard procedures.[3]

Results and Discussion

The bacterial counts per gramme of different meat products are summarised in Table 1. The average total viable count for the surface washings ranged from 10×10^6 to 40×10^6/ml, whereas for the blended samples the range was from 1×10^5 to 30×10^6/g of sample. The data for the viable counts of different bacteria in ready-to-cook products are shown in Table 2. The details of the microorganisms isolated and confirmed upto species level and classified in three groups are indicated in Table 3.

From Table 3 it is evident that 42.53 per cent of the microorganisms belonged to spoilage type, 7.22 per cent

TABLE 1. TOTAL VIABLE COUNTS (TVC) OF VARIOUS READY-TO-COOK PORK PRODUCT SAMPLES

Name of sample	No. of samples	Mean IVC of surface washings/ml $\times10^6$	Range of TVC of blended sample/g	Mean TVC of refrigerated sample plates $\times10^5$
Cocktail sausages	29	10	1×10^5 to 4.5×10^6	—
Oxford sausages	16	10	8×10^5 to 5×10^6	5.5
Porkies (pork and breakfast) sausages	16	20	4×10^5 to 9×10^6	—
Ham (cured)	8	20	3×10^5 to 1×10^6	4
Kababs (mutton and pork)	16	40	5×10^5 to 30×10^6	—
Bacon (cured)	10	10	3×10^5 to 3×10^6	—

TABLE 2. MEAN OF BACTERIAL (DIFFERENTIAL COUNT) COUNTS PER GRAMME OF BLENDED PRODUCTS

Bacteria	Cocktail sausages $\times 10^5$	Oxford sausages $\times 10^5$	Porkies $\times 10^5$	Ham $\times 10^5$	Kababs $\times 10^5$	Bacon $\times 10^5$
Staph. aureus	2	3	6	6	3	5
Micrococcus sp.	2	2	2	3	2	3
Bacillus sp.	3	5	3	3	5	3
E. coli	2	4	4	4	1	2
Klebsiella	1	—	4	1	2	2
Serratia	2	—	—	—	7	—
Lactobacillus	—	3	—	2	—	2
Strep. fecalis	1	—	2	2	4	1
Clostridium (Sulphite reducing)	—	—	—	—	3	4
Pseudomonas	—	—	—	—	—	1
Salmonella	—	—	2	—	3	—

TABLE 3. MICROORGANISMS ISOLATED FROM DIFFERENT TYPES OF PORK PRODUCTS

Name and type of organisms	Pork products and number of isolates: Cocktail sausages (29)	Oxford sausages (16)	Porkies (16)	Hams (8)	Bacons (10)	Kababs (16)	Total isolates
Spoilage types:							
Staph. epidermidis	9	7	—	5	5	9	35
M. luteus	4	5	3	—	—	3	15
Micrococcus sp.	4	—	4	4	4	6	22
B. subtilis	10	—	5	—	—	—	15
B. megaterium	—	—	—	—	5	4	9
Bacillus, sp.	3	3	4	3	4	6	23
Proteus vulgaris	10	4	6	1	3	—	24
Proteus rettgeri	—	—	—	—	—	6	6
Klebsiella aerogenes	6	—	3	2	5	—	16
Total							165
Potential spoilage types:							
Sarcina lutea	—	—	—	8	3	—	11
Gaffkya tetragena	—	—	—	4	—	—	4
Brevibacterium linens	1	—	—	—	—	—	1
Brevibacterium fulvum	—	—	—	2	—	—	2
Lactobacillus brevis	—	2	—	3	2	—	7
Achromobacter guttatus	—	2	—	1	—	—	3
Total							28
Pathogenic types:							
Stap. aureus	19	8	13	5	9	7	61
Strep. faecalis	2	—	7	2	8	10	29
Cl. perfringens	—	—	—	—	6	3	9
B. cereus	8	7	6	1	6	9	37
Serratia marcescens	1	—	—	—	—	3	5
E. coli	1	8	7	2	4	6	27
Proteus mirabilis	—	5	—	—	4	3	12
Klebsiella pneumoniae	—	—	—	—	—	4	4
Pseudomonas aeruginosa	—	—	—	—	1	—	1
Salmonella enteritidis	—	—	3	—	—	7	10
Total							195

Figures in parenthesis indicate the total number of samples processed.

were of potential spoilage type and 50.26 per cent were of pathogenic type.

Meat products containing pork comprise a larger group of ready-to-cook foods than other meat products. Pork spoils faster than other meats held under similar conditions. Owing to the hot and humid climate of Bombay these products were thought to have a larger number and variety of microorganisms present in them, than other meat products. Organisms such as the *Staph. aureus*, *Staph. epidermidis*, *Micrococcus* spp. *Strep. faecalis*, *Bacillus cereus*, *Klebsiella aerogenes*, *E. coli*, *Achromobacter* spp. *Pseudomonas* spp. are known to survive the refrigeration, as well as deep freezing temperatures under favourable conditions[6,7]. Most of these organisms being proteolytic in nature, eventually cause spoilage of meat preparations in which they proliferate. They are encountered commonly in fresh raw meats, pork sausages[8,9]. bacons, continental sausage meats[10] and hams[11].

Staph. aureus was isolated invariably from all the food samples which indicated that they can survive low storage temperatures[7]. Further, these products contained sodium nitrate, which also had no inhibiting effect on the growth of the *Staphylococci*[12]. The viable count ranged from 2×10^5 to 6×10^5. An average viable count of 5.8×10^3 *Staphylococci* was reported by Hall *et al*[13].

In the present study, the isolation of the *Micrococcus* sp. from the pork sausages[8], the cured[10] and ready-to-cook meat products stored under refrigeration conditions and having an average viable count of 2.3×10^5, indicates their survival capacity under favourable conditions.

Strep. faecalis were commonly encountered during the present study, with an average viable count of 2.2×10^5. They are the "indicator[5]" organisms of faecal contamination. Therefore, their presence is objectionable as these indicate inadequate hygiene in the processing plant.

Sulphite reducing *Cl. perfringens* were encountered in Kababs and Bacon, having average total viable counts of 3×10^5 and 4×10^5 respectively, which is lesser than 10×10^5 required to cause food poisoning in man[14]. Isolation of various species of *Bacillus* group indicates their ubiquitous occurrence and the tedious task facing the manufacturers in getting rid of them from the food. These species are reported to cause putrefactive spoilage[8,9] of meat products. *Cl. perfringens* and *B. cereus* are generally indicative of extraneous contamination and inadequate heat treatment[15], they are of importance as they are responsible for causing food poisoning incidences. In general 50 per cent of all meats and meat products are contaminated with spores of *Cl. perfringens*[14].

The isolation of *Klebsiella pneumoniae* from Kababs is suggestive of contamination of these pork products by infected human beings at some stage in food processing.

The total viable count of *E. coli* ranged from 1×10^5 to 4×10^5, this finding is in agreement with those of Ayres[8] and Kitchell[10]. *E. coli* and *Coliforms* are indicative of sewage contamination at some stage during processing, and may cause gastroenteritis, if enteropathogenic variety of *E. coli* are present[16].

The presence of *Salmonella* in the Porkies and the Kababs is of zoonotic importance, as the major ingredient in these products was pork. Their average viable counts were 2×10^5 and 3×10^5 respectively. Their occurrence therefore, either means that there was initial contamination of the raw pork used[17], or that contamination occurred during processing, through the casings used[18]. These *Salmonella* are also responsible for causing mild to fatal cases of food poisoning[19].

During the present study 42 *Proteii* isolates were obtained, of which 12 were of *P. mirabilis*. All *Proteii* cause putrifactive spoilage of foods.

Although *Serratia marcescens* is known as a spoilage organism[8], its presence is more of aesthetic significance, since it is responsible for the discolouration of food products. It was encountered in cocktail sausages and Kababs with an average viable count of 2×10^5 and 7×10^5 respectively. It produces an endotoxic substance, responsible for urinary tract and pulmonary infections, ranking next to *E. coli*.

The isolation of *Pseudomonas aeruginosa* from bacon having a viable count of 1×10^5 is indicative of contamination during packaging. Apart from being a psychrophilic meats spoilage organism, it is also a source of food poisoning[20].

Achromobacter sp. encountered in Oxford sausages and ham with an average viable count of 5.5×10^5 and 4×10^5 respectively is attributed with the souring of refrigerated hams[11] and spoilage of fresh pork sausages[8],

In these studies several bacteria harmful to the consumer's health or public health in general were encountered. A bacteriological control of meat foods is nearly non-existant in our country at present, since it has not been imposed legally upon the manufacturers, as it has been done in other countries. As a result of which not only do the consumers suffer from public health point of view, but also the exporters of these products incur heavy economic losses, due to non-fulfillment of the bacteriological standards of importing countries. Therefore, a bacteriological examination is essential in our country.

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Control of Fungal Stem-End Rot *(Thielaviopsis Paradoxa)* During the Transport of Pineapples

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Stem-end rot caused by *Thielaviopsis paradoxa* in pineapples is a principal source of spoilage of the fruit. Earlier reports indicate that this spoilage could be controlled with fungicidal treatment. In the present study, where a trial lorry load of pineapples, packed in bamboo basket was transported from Agartala to Calcutta, with a transit period of 4½ days, it was observed that dusting of the stem-end with a small amount (0.1 g percent) of benzoic acid with an inert substance such as kaolin, was highly effective in controlling the stem-end rot and was also commercially feasible on account of the low cost of the treatment.

The extent of spoilage of pineapple during transport is dependent upon the variety, maturity stage, duration of transport, climatic conditions, etc. Spoilage as high as 50 per cent has been reported in pineapples sent from Tripura to Calcutta after onset of the monsoon in the region.

There are different types of spoilage such as lateral rot, core rot or soft rot which is also known as stem-end rot. The latter is caused by the fungus *Theila viopsis paradoxa* during transport. Treatment with fungicides such as benzoic acid, salycilanilide, benomyl, has been reported to control this spoilage[1-4].

In the present study, the commercial feasibility of controlling the stem-end rot by using benzoic acid has been investigated.

Materials and Methods

Pineapples of the 'Queen' variety weighing 600 to 1000 g in advanced maturity (grade A) with the eyes and the adjoining bracts of the bottom half of the fruit turning pink, were harvested and kept in bucket shaped bamboo baskets measuring approximately top diameter 20 in; bottom diameter, 14 in; and depth, 14 in;. About 30-35 fruits were packed in each basket; dry sun

grass was used as cushion around the periphery of the baskets. The treated and control fruits were arranged one above other, the crown facing downwards, and transported from Agartala to Calcutta market in June 1977. Monsoon commenced during the first week of june and the fruits were harvested on 14th June. The fruit baskets were stacked in three tiers in the lorry, keeping equal number of baskets of each treatment and control in front and rear portion of the lorry. Lids were stitched onto these baskets before arranging them in the lorry. The lorry, carrying the fruit baskets left Agartala at 9.00 p.m. on 14th June and reached Calcutta in the morning of 19th June, the transportation period being 4½ days.

Treatment: The fungicide was prepared in two ways. (*i*) Benzoic acid (1 part) was mixed with inert Kaolin (4 parts) and (*ii*) in the form of alcoholic solution. A 2:5 percent solution of benzoic acid (w/v) was prepared in 30 percent alcohol. The dust or the solution was applied to the cut stem-end by means of a cotton pad, within 6 hr after harvest. There were 1,200 fruits for each of the treatments and an equal number of fruits were kept as untreated control. In each case, 30-35 fruits were packed, in each basket. There were 35 baskets in each of the two treatments while 45 baskets were kept as control.

Loading: Half the number of the baskets were stacked in front portion of the lorry and half in the rear portion, the total number being 115 baskets. The space left on the top of the stacked baskets was loaded with unpacked and untreated pineapples to make up the lorry load as in the case of the conventional practice; taking care not to allow the load of these pineapples to rest on the fruits in the baskets by keeping bamboo Knitted sheets (*thatti*) re-inforced with bamboo battens supported on the side walls of the lorry.

At Calcutta 36 baskets were unpacked at random from each of the three treatments and examined in for spoilage of different kinds. The spoilage causing fungus was isolated and identified as per the procedure described by John Tuite[5] and Ellis[6,7].

Results and Discussion

Data regarding the effect of fungicidal treatments on the spoilage, specific as well as general, are given in Table 1. Spoilage occurred in different parts of the fruit. There was stem-end or core rot caused by *Thielaviopsis paradoxa* and also crown rot. There was also lateral rot caused mostly by *Aspergillus* sp. and *Pencillium* sp. In some cases, both lateral and core rots were observed. The former presumably through the bruised portion and the latter through the stem-end of the fruit or the crown. Spoilage on account of stem-end rot was found to be 5.0, and 9.2 percent in fruits treated with benzoic acid dust, and alcoholic solution respectively, whereas in control it was 34.3 per cent. The lateral rot was not controlled by the fungicidal treatment. Incidence of crown-rot was comparatively less than the other two types. This might be due to the fact that in the package there was much chance of the crown of the one fruit coming in contact with the stem of another fruit, thereby spreading infection by contact which was

TABLE 1. EFFECT OF FUNGICIDAL TREATMENT ON CONTROL OF SPOILAGE IN PINEAPPLES (VARIETY: QUEEN) DURING TRANSIT FROM AGARTALA TO CALCUTTA (JUNE 1977)

Treatment	Stacking position in lorry	Stem-end rot (%)	Lateral rot (%)	Crown rot (%)	Total rot (%)	Total* spoilage (%)
Benzoic acid dust (T_1)**	Front	0.4	5.6	1.4	7.4	7.0
	Rear	9.6	15.9	1.0	26.5	18.0
	Mean	5.0	10.7	1.2	17.0	12.5
Benzoic acid in alcohol (T_2)**	Front	10.9	21.9	4.4	37.2	24.0
	Rear	7.5	13.9	5.0	26.1	16.9
	Mean	9.2	17.8	4.7	31.7	20.4
Control (T_3)	Front	33.6	16.5	8.7	58.8	46.2
	Rear	35.0	20.4	9.5	64.9	50.0
	Mean	34.3	18.5	9.1	61.9	48.1

*The values are less than the total rot because in some fruits, more than one type of spoilage occurred.

**The difference between T_1 and T_2 over control (T_3) were significant at P = 001 levels for stem-end rot.

arrested. In general, application of benzoic acid in the form of dust was highly effective in the control of stem-end rot under the experimental conditions of the treatment, packaging and transport.

Commercial feasibility of the treatment: It took nearly 60 and 90 min for a worker to treat 600 fruits with the dust and alcoholic solution respectively. The workers however, preferred the dusting operation. Using the dust form of application and taking into account the present cost of benzoic acid and kaolin being Rs. 60 and Rs. 2.50/kg respectively, the material cost for treating 10,000 fruits (one lorry load) is estimated as Rs. 14. One kilogram of the blended dust (1:4) was sufficient for treating 10,000 fruits. The cost involved for labour is separate. This shows that there is scope for using benzoic acid for control of the stem-end rot in pineapple fruits during transportation. However, remedial measures for controlling other types of rots also must be explored.

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Effect of Freezing Methods on the Quality of Freeze Dried Alphonso Mangoes

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The influence of freezing methods on the textural characteristics of 'Alphonso' mangoes were determined. The mango slices were subjected to the following treatments! freezing at-20°C in a deep freezer using a contact plate freezer (-40°C) or by quick freezing by immersing in liquid nitrogen (LN_2) (-196°C) and freeze dried to 2% moisture content. After reconstitution, the samples were tested for (i) rehydration properties, (ii) water holding capacity (WHC), (iii) textural evaluation by means of shear press and (iv) organoleptic scoring. Results indicate that freezing temperature is the most critical factor affecting the cell structure of mango. Freezing at -20°C or at -40°C results in considerable disruption of cellular structure, whereas it was minimal at -196°C. Slices frozen at -196°C showed firmer structure as well as higher water holding capacity than the remaining samples frozen by slow and moderate freezing.

Recent innovations have resulted in more rapid freezing of foods along with improvement in their texture[1]. The rate of freezing is recognised as a critical factor in tissue damage and the conventional slow freezing of fruit and other multicellular structures is often harmful[2]. Improved texture has been associated with faster freezing rates[3], which may be related to the formation of smaller size of the ice crystals and their uniform distribution. Brown[4] showed that rapid freezing in LN_2 resulted in less tissue damage and consequently better texture than conventionally frozen beans. The present work describes the effect of freezing temperature and hence freezing rate, on the textural quality of freeze-dried 'Alphonso' mangoes.

Materials and Methods

Fully ripened 'Alphonso' mangoes available from the local market were brought to the laboratory. The fruits were washed, peeled manually using stainless steel knives, sliced into blocks of $20 \times 15 \times 10$ mm and then divided into three batches for different freezing treatments.

Freezing methods: Three freezing methods were employed (*i*) Mango slices were spread on stainless steel trays and were frozen (TF) in a deep freeze (–20°C); (*ii*) Contact plate freezing (–40°C) (CPF) by freezing the samples while in contact with two freezing plates; and quick freezing (QF) by immersing in a wide mouth polystyrene flask containing liquid nitrogen.

The temperature measurements were made by inserting a copper-constantan thermocouple probe at the centre of the sample.

Freeze drying: The samples frozen by the above three freezing methods were dried in a B.A.R.C. model freeze-drier as described by Ramamurthy *et al*[5]. to 2 per cent moisture content. The drying cycle was 20 hr for all the samples.

Moisture content: Moisture content was determined by AOAC method[6] by drying the freeze dried samples in triplicate in a vacuum oven.

Rehydration: In order to determine the reconstituability, the weighed freeze dried samples were immersed in large excess of water at 26±2°C. At 2 min intervals during the first 6 min and later on at 5 min intervals, samples were removed, drained on a 8-mesh screen for 1 min and then reweighed. The amount of water absorbed/g of freeze dried material was calculated upto 30 min of soaking.

Water holding capacity: This was measured by centrifuging fresh or rehydrated mango slices for 5 min at 500, 1000, 1500 and 2000 rpm respectively in a Sorvall centrifuge. The centrifuge cup was weighed and weight loss of the mango slice was calculated.

Shear analysis: The texture of mango slices was measured using an Instron Universal RTesting Machine equipped with an integral strip chart recorder. Force was applied by a flat ended 4 mm dia. probe placed on the slice, The force (G) required to shear the slice 5 mm was considered as indicative of the firmness of the mango when the recorder showed a sharp peak.

Organoleptic evaluation: Organoleptic characteristics of the samples were determined using a 9-point hedonic scale based on colour, odour and texture. After reconstituting in distilled water, samples were served to a panel of six individuals along with control sample. The results were expressed by: 9 representing "excellent" quality, 5 indicating fair and 1 representing "poor". The average score was computed from the panel results.

Results and Discussion

Freezing times: The time required for the mango slices to reach −10°C varied from 40 sec to 12 hr when different freezing methods were used (Table 1).

TABLE 1. METHODS OF FREEZING MANGO SLICES PRIOR TO FREEZE DRYING

Rate	Methods of Freezing	Temp (°C)	Time
Fast	Immersion in liquid N	−196	40 sec
Moderate	Contact plate freezing	−40	2 hr
Slow	Tray freezing	−20	12 hr

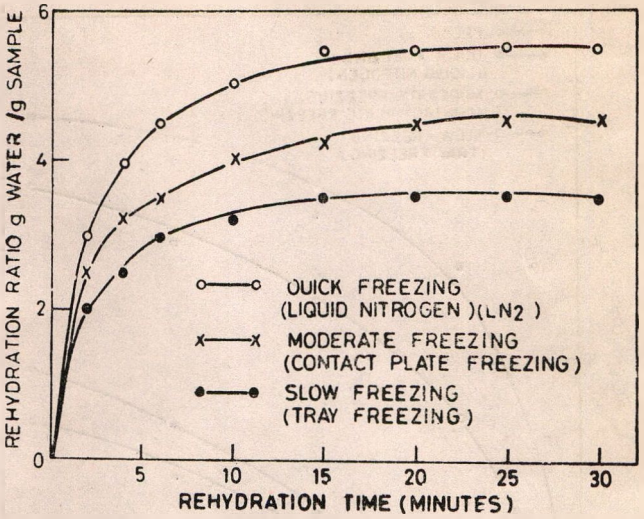

Fig. 1. Effect of freezing methods on rehydration characteristics of freeze dried mangoes.

Rehydration studies: The rehydration properties of freeze dried mangoes frozen by different freezing methods are shown in Fig. 1. Water uptake in freeze dried mangoes was more rapid during the first few minutes. A rehydration ratio of 5.0 was obtained for the sample frozen by quick freezing, after 30 min. The samples frozen by tray freezing (TF) and contact plate freezing (CPF) showed rehydration ratios of 3.5 and 4.5, respectively.

Water holding capacity (WHC): The influence of freezing rate on the water holding capacity of freeze dried mango is shown in Fig. 2. Weight loss in rehydrated freeze dried mangoes at four centrifugal speeds ranging between 500 and 2000 rpm occurred in increasing order with increasing rpm in all the freezing methods. The percentage weight loss varied between 25 and 55 in the different freezing methods.

Shear analysis: The shear press data for freeze dried mango slices frozen by different freezing methods is shown in Fig. 3. The quantitative measurement of textural properties, as determined by shear press test, showed that freezing in LN_2 showed highest shear value of 750 g and was close to that of the fresh mango (800 g). The shear values for the contact plate frozen and tray frozen samples were 600 g and 350 g respectively.

TABLE 2. ORGANOLEPTIC SCORE OF FREEZE DRIED MANGO FROZEN BY THREE DIFFERENT METHODS OF FREEZING

Fresh	Quick frozen (LN_2)	Contact plate freezing	Tray freezing
9	8-9	7	5-6

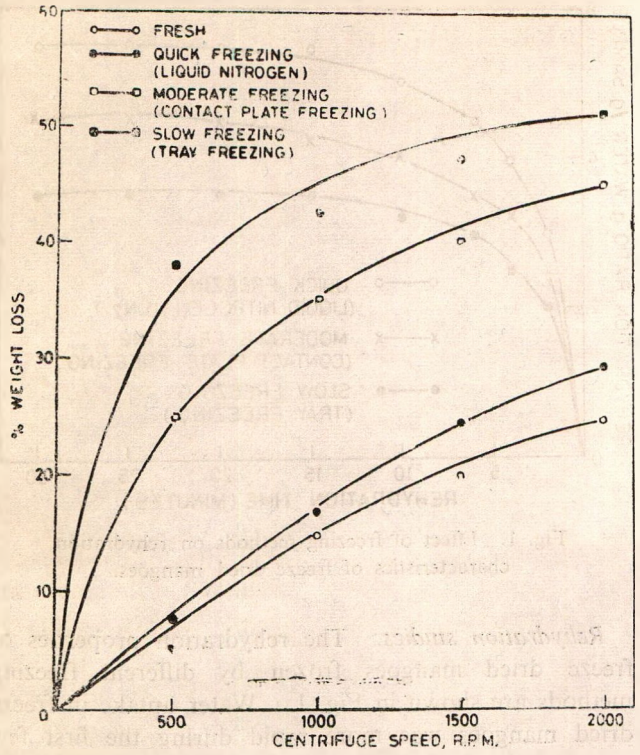

Fig. 2. Weight loss in freeze dried mangoes as influenced by freezing parameters and determined by centrifugation.

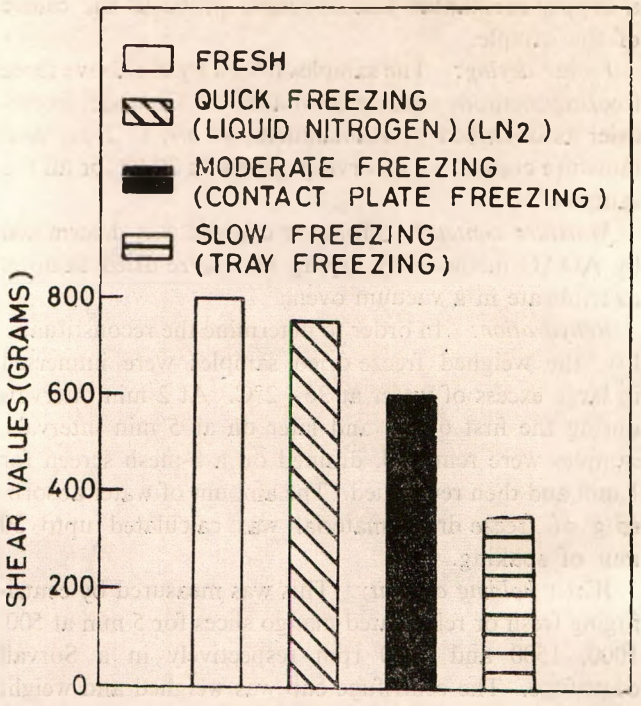

Fig. 3. Texture of freeze dried mangoes as affected by methods of freezing as measured by Instron Universal testing machine.

Organoleptic evaluation: The samples frozen by LN_2 prior to freeze drying had higher ratings (8-9), whereas the slow frozen ones had scores of only 5-6 for TF and 7-8 for CPF. (Table 2).

Thus, the rate of freezing of mango slices has been shown to have a significant influence on textural characteristics. This confirms reports by Sterling[7], Jacobson[8], and Bannister[9] who observed that freezing rates had considerable influence on textural characteristics of tissues of apple, meat and pork. The present results show that freezing caused a notable change in the textural properties of freeze dried 'Alphonso' mango slices. A decrease in the rate of freezing caused an increase in fluid loss and greater loss of textural properties as measured by shear press values. The freezing rate considerably alters the rehydration and organoleptic properties of the freeze dried mangoes. These findings are in agreement with those on poultry meat[10], tomato slices[11], and mushroom[12] and suggest that rapid freezing preserves the integrity of muscle tissue to a greater extent than slow freezing.

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Some Quality Aspects of a Few Varieties of Cassava

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Physico-chemical, processing, and edible characteristics of five selected hybrid varieties of cassava were studied. They showed considerable difference in some of the above properties. There were varietal differences in starch recovery, and cyanide contents. Increase in cyanide content in the tubers was associated with a bitterness in taste even after cooking. 'M-4' variety which had the highest starch content, showed a high degree of stickiness as cooked flour. The tubers showed considerable differences when cooked and evaluated in different forms.

Cassava (*Manihot esculenta* Crantz) is indigenous to tropical regions. Factors such as variety of the plant, stage of maturity, agronomic conditions, fertilizer stage of harvesting etc. largely determine the yield and quality of the tuber[1-3]. Techniques such as intervarietal hybridisation[4], application of hormone like Seradix B Powder[5] have been found to improve the yield and rooting of the tuber. Recently Nayar[6] showed the possibility of improving the quality of the tuber by mutation breeding.

A large number of studies reporting variations in composition have been reported by several workers[7-10]. In this paper, results of a comparative study of the varieties of cassava, namely, 'M-4', 'H-165', 'H-2304', 'H-226' and 'H-1687' are presented.

Materials and Methods

The hybrid varieties, 'H-165', 'H-2304', 'H-226' and 'H-1687' were procured from Central Tuber Crops Research Institute and 'M-4' from the local market.

Fresh tubers were peeled, washed and cut into chips of 5 mm thickness by using a hand slicer. The chips were divided into three lots and used individually for parboiling, preparation of flour and starch.

Flour: The chips were dried in a cross flow drier at about 60±2°C and then powdered into flour.

Parboiled chips: Fresh chips were parboiled by immersing in boiling water for 10 min, draining followed by drying as above.

Starch: Fresh chips with extra water were crushed in a waring blender and contents filtered through cloth. The residue was again extracted with water in the blender and starch filtered. This was again repeated with the residue and the remnants. The starch was allowed to sediment at room temperature for 4-6 hr and then in the refrigerator overnight. The sediment was washed with water after draining. The starch residue was spread and dried first at room temperature and later at 50-55°C.

Analytical: Starch, protein, crude fibre, total and insoluble ash, fat and HCN contents were determined according to the AOAC methods[11]. The digestibility of flour was studied according to the procedure described by Modi and Kulkarni[12]. The pH of the aqueous extract (1:1, w/v) was measured with a Beckman pH meter. The colour of the flour was read in a reflectance meter.

The material was prepared in three ways for acceptability tests: (*i*) freshly cooked, (*ii*) parboiled and fried in coconut oil, and (*iii*) steam cooked with 1.5 per cent salt. The products were evaluated by a selected panel of judges for texture, colour, flavour and general acceptability from highly undesirable to highly desirable.

Results and Discussion

The recovery of starch was more in the case of 'H-226' and minimum in 'H-165' (Table 1). The dry matter content also showed variation among cultivars. 'M-4' variety showed maximum dry matter content. Intervarietal difference had very little effect on the digestibility values. This was ascribed to a narrow range amylase-activity values ranging from 65.2 to 72.5. Peeling loss (14.5 to 15.9 per cent) and pH values (6.2 to 6.8) were more or less same in all the varieties. There was considerable difference in the cyanide contents of the freshly peeled varieties among which 'H-165' rated as most bitter, even after cooking. This variety had the highest amount of HCN. Tubers with cyanide contents could only be used for the manufacture of industrial starch-derived products like adhesives.

TABLE 1. PHYSICO CHEMICAL CHARACTERISTICS OF FRESH CASSAVA VARIETIES*

Variety	Dry matter (%)	Peeling loss (%)	Starch recovery (%)	Fibrous residue (%)	HCN (mg/100 g)	pH	Digestibility (mg maltose/100 g)
M-4	40.7	14.4	23.3	6.2	<10.0	6.8	68.4
H-2304	32.6	15.6	22.0	6.0	16.2	6.6	72.5
H-1687	33.4	12.6	20.5	4.2	10.0	6.8	68.8
H-165	34.1	14.5	19.5	3.5	27.6	6.6	68.8
H-226	33.9	15.9	24.7	5.8	11.3	6.2	65.2

*Average value obtained for 10 samples of each variety.

Total starch content was highest in 'M-4' variety and lowest in 'H-165' (Table 2). An inverse relationship between starch and fibre contents was also obtained. A positive corrleation between starch and dry matter content reported by Jong[13] was, however, not observed in this study. 'H-1687' and 'H-165' were less whitish in appearance than rest of the samples. The same was shown in their reflectence values[14]. Ash and acid insoluble ash were low in all the samples. While the low fibre content helps the use of flour as food, its low protein content makes it necessary to mix with high protein flour for use as a balanced staple food.

Fried products from parboiled chips of all the varieties were satisfactory in flavour (Table 3). Though chips from 'H-2304' were hard, all were found to be crisp in texture, Steam cooked products from commercial 'M-4' variety were found to be least satisfactory because of excessive stickiness. This could possibly be due to its high starch content. As freshly cooked tubers 'M-4' variety ranked highest in texture and taste. All the hybrid varieties were rated as "slightly unsatisfactory" in taste. The hybrid variety 'H-2304' alone was rated "desirable" in texture.

TABLE 2. COMPOSITION OF CASSAVA FLOUR

Variety	Ash (%)	Acid insoluble ash (%)	Crude fibre (%)	Starch (%)	Fat (%)	Protein (%)	Reflectance (%)
M-4	2.04	—	1.50	86.0	0.38	1.42	98
H-2304	1.50	0.069	2.20	85.1	0.36	2.62	98
H-1687	2.43	0.058	2.23	80.5	0.17	2.86	94
H-165	2.09	0.007	2.98	79.1	0.56	1.31	90
H-226	2.13	0.067	2.13	81.7	0.59	1.54	96

TABLE 3. ORGANOLEPTIC EVALUATION OF PRODUCTS OF DIFFERENT VARIETIES OF CASSAVA

Variety	Colour after peeling	Cooking quality		Frying quality of parboiled chips			Steam cooked products (flour)		
		Texture	Taste	Colour	Crispness	Flavour	Stickiness	Taste	Acceptability
M-4	Whitish	10	10	Light brownish	Crisp	Good	2.8	6.8	4.8
H-2304	Creamish	5.4	5.2 (S.B.)	Brownish	Crisp and hard	Fair	5.1	6.0	5.2
H-1637	Yellowish cream	6.6	4.9) (B)	Brownish	Crisp	Satisfactory	4.4	5.4	4.9
H-165	Creamish white	5.6	4.7 (V.B.)	Light brownish	Crisp	-do-	3.4	2.8	3.1
H-226	Whitish	5.2	5.2 (F)	Brownish	Crisp	-do-	5.2	5.6	5.3

Score: 0-4, highly undesirable; 4-6, slightly unsatisfactory; 6-8, desirable; 8 and above, highly desirable

S.B. slightly bitter; B. bitter; V.B. very bitter; F. fibrous.

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Electrophoretic and Solubility Characteristics of Proteins in *Phaseolus mungoreous (Phaseolus aureus* Var. T_1 x *Phaseolus mungo* Var. M_{1-1})

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Analysis of protein fractions showed that the *Phaseolus mungoreous* contained the least amount of globulin fraction (1.46%) as compared to parents, *Phaseolus aureus* (2.20%) and *Phaseolus mungo* (1.49%). Remarkable variations were, however, noticed in the relative proportions of these fractions which range in percent from 6.13 in *P. mungo* to 9.98 in *aureus* for albumins; from 1.46 in the *mungoreous* to 2.20 in *aureus* for globulin; prolamine and glutelin varied from 0.53 to 0.91 and 4.20 to 11.09 respectively. Separation by polyacrylamide gel electrophoresis reveals a diversity of protein bands amongst the species. Maximum number of 19 bands, are obtained in *aureus* and 18 in *mungo* with 12 in *mungoreous*. A marked difference in protein pattern within these pulse species is clear from the scanning of protein bands in anionic system, scanned by Beckman spectrophotometric scanner at 600 nm.

The pulses have high content of protein, even greater content of carbohydrate and a low level of oil content[1]. Salt soluble globulins are the main proteins in green gram seeds[2]. It was also reported[3] on the basis of extracted non-dialysable nitrogen in safflower protein (*Carthamus tinctorius* L.) that 18.1 per cent was water soluble, 41.5 per cent salt soluble and 39.1 per cent alkali soluble. Data on protein fractions of the legume proteins have provided additional information on the nutritive value[4] and the globulin has been referred to as Jaffe's bean globulin since it is particularly resistant to digestion[5-6].

The yield, protein content and protein quality of pulses could be improved by breeding[7]. The hybrids contained protein[8] from 21.56 to 25.68 per cent. Only three crosses viz. P-23-67 × Jawahar 45; Jawahar 45 × No 305 and Jawahar 45 × hybrid 4, contained protein above 25 percent. In this University a cross between black gram (*Phaseolus mungo* var. M_{1-1}) and green gram (*Phaseolus aureus* var. T_1) which is called as *Phaseolus mungoreous* (Amphidiploid) has been developed. In the present investigation, this cross has been examined for protein component and compared with those of the parents.

Materials and Methods

Samples of black gram (*Phaseolus mungo*) var. M_{1-1}, green gram (*Phaseolus aureus*) Var. T_1 and their cross, *Phaseolus mungoreous* (amphidiploid) were obtained from the Department of Genetics, of this University.

Samples of each variety were ground to pass through 100 mesh sieve and stored in air tight containers.

Different fractions, viz., albumin (water soluble), globulin (salt soluble), prolamine (alcohol soluble) and glutelin (alkali soluble) ware separated by the method of Osborne and Mendal as modified by Naik[9]. The nitrogen content in these fractions was determined[10,11] and expressed as protein (Nitrogen per cent×6.25).

Polyacrylamide disc electrophoresis was employed to separate the soluble proteins, because of its high resolving power, transparency and chemical inertness. Anionic system of disc acrylamide gel electrophoresis was used for separating the different proteins. The concentration of the gel used was 7.5 percent.

Total proteins were extracted from the samples in 0.05M tris buffer, pH 7.6 containing 5μ mole β-mercaptoethanol.

Electrophoresis was conducted in cold for about 90 min at 5 mA current per gel column. After completion of electrophoresis, gels were removed and stained in one per cent amido black in 7 per cent acetic acid and destained by diffusing in 7 per cent acetic acid. Rf of each band was determined by comparing against reference dye. Gels were scanned at 600 nm with the help of scanner using Beckman Spectrophotometer.

Results and Discussion

Different protein fractions i.e. albumin, globulin, prolamine and glutelin contents of these varieties of pulses are reported in Table 1. It is evident from the data that the true protein is high in the *P. mungoreous* 22.35 as compared to its parents *P. mungo* 20.58 and *P aureus* 17.01, The values (percent) for albumin fraction ranged from 6.13 to 9.98; globulin from 1.46 to 2.20; prolamine from 0.53 to 0.91 and glutelin from 4.20 to 11.09. The data further indicate that albumin, prolamine and globulin contents were comparactively high in parents, whereas glutelin content is high in cross and low in parents. Liener[5] showed that globulin protein is resistant to digestion. Thus the cross should provide a nutritionally better profile than that of the parents since the protein resistant to digestion is decreased. Thus it is singularly important for the breeders to look for specific changes in protein fractions in the beans rather than the gross protein contents.

Rf values of soluble proteins of different varieties of pulses separated by gel electrophoresis is presented in Table 2. The pattern of proteins from different variety of pulses in anionic system, scanned by Beckman spectrophotometer scanner at 600 nm are shown in Fig. 1

TABLE 2. Rf VALUES OF SOLUBLE PROTEINS IN *MOONG, MASH* AND THEIR CROSS

Phaseolus aureus (T_1) (Moong)	*Phaseolus mungo* (M_{1-1}) (Mash)	*Phaseolus mungoreous* (Cross)
0.17	0.17	0.17
0.21	0.21	—
0.28	0.28	—
0.30	—	—
—	0.34	0.34
0.36	0.36	—
0.42	0.42	—
0.44	0.44	0.44
—	0.47	—
0.50	0.50	0.50
—	0.52	—
—	0.55	0.55
0.57	—	—
0.58	0.58	0.58
0.65	0.65	0.65
0.68	0.68	—
0.73	0.73	0.73
0.77	0.77	0.77
0.80	0.80	0.80
0.85	0.85	0.85
0.89	—	—
0.95	—	0.95
0.97	—	—

TABLE 1. DIFFERENT MAJOR PROTEIN FRACTIONS IN *MASH* AND *MOONG* VARIETIES AND THEIR CROSS

Varieties	Albumin %	Globulin %	Prolamine %	Glutelin %	Total proteins %
Mash (*Phaseolus mungo* M_{1-1})	6.13	1.49	0.91	10.06	18.59
Moong (*Phaseolus aureus* T_1)	9.98	2.20	0.53	4.20	16.91
Cross (*Phaseolus mungoreous* amphidiploid)	9.01	1.46	0.76	11.09	22.32

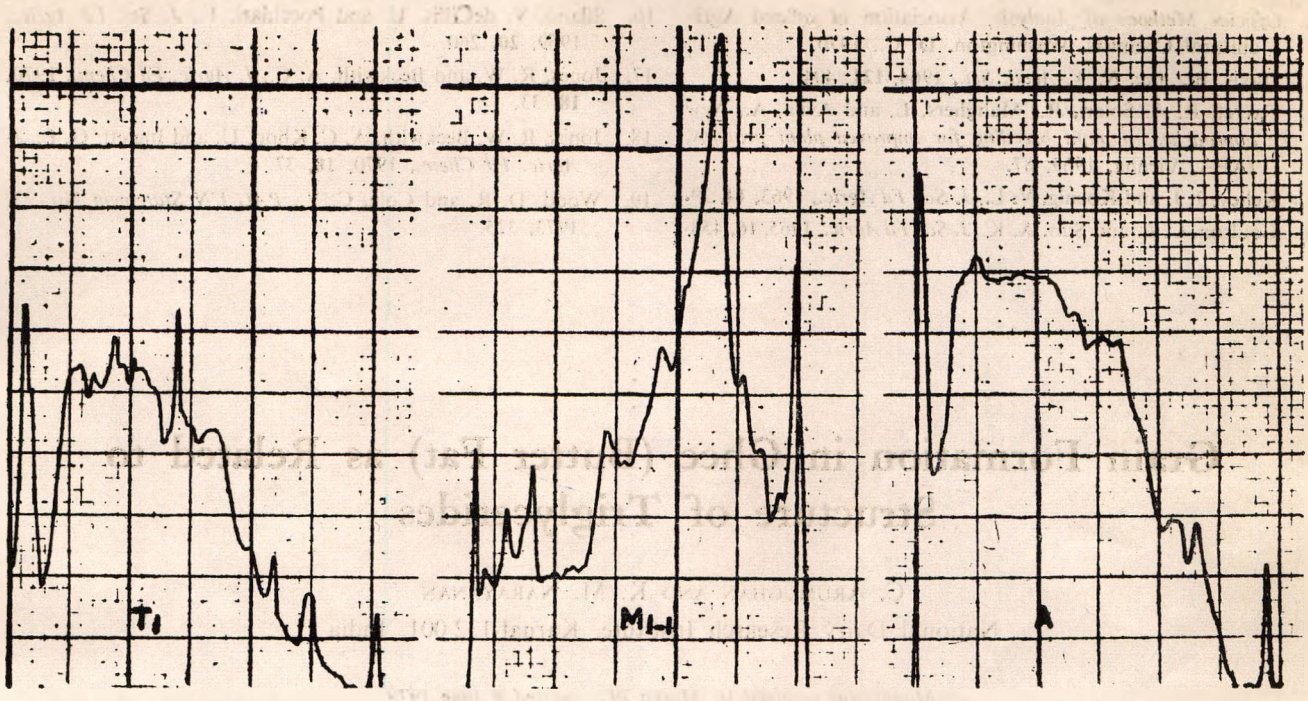

Fig. 1. Scann of protein bands of various varieties (*Phaseolus aureus* T_1, *Phaseolus-mungo* M_{1-1} and a cross Amphidiploid A) separated by acrylamide gel electrophoresis.

In anionic system, bands of Rf values of 0.17, 0.44, 0.50, 0.58, 0.65, 0.73, 0.77, 0.80, and 0.85 are common in all the varieties whereas bands of Rf values of 0.21, 0.28, 0.36, 0.42 and 0.68 are observed only in parents i.e. *moong* and *mash*. Similarly bands of 0.34 and 0.55 Rf values can be seen only in amphidiploid and *P. mungo* and another 0.95 Rf value is detected in *P. aureus* and amphidiploid, while bands of Rf values of 0.30, 0.57, 0.89, and 0.97 are observed only in *P. aureus* and bands of 0.47 and 0.52 Rf values are observed only in *P. mungo*.

One line of investigation in exploring the nutritive value of beans as a human food is to examine the component seed proteins that are characteristics of the species. Separation by Polyacrylamide gel electrophoresis reveals a diversity of protein bands from one species to another. As pointed out by Favret *et al*[21] the presence or absence of particular band may be shown to be under genetic control.

In Fig. 1. the number of peaks in scan coincide with the number of bands observed. A marked difference in protein pattern within these pulse species is again clear from the scan of protein bands separated by polyacrylamide gel electrophoresis. Maximum number of bands viz., 19 are obtained in *P. aureus* and 18 in *P. mungo* and 12 in the cross (*P. mungoreous*). These results show species differences in the protein make up of different pulses.

Other workers[13-18] have also reported that differences in species are reflected in protein make up. Wood and Cole[19] have pointed out that knowledge of the bands of bean seed proteins and their nutritional characteristics could be the basis of a very precise screening system. Genotype would be screened for the quality and the number of the seed proteins present and the nutritional value could be predicted based on the knowledge of the protein components. The plant breeders should carry the predictive process a step further, knowing the extent of the genetic variations for seed proteins. They could predict the genotype of the bean cultivar that could be developed with protein of the higest possible nutritional value.

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Grain Formation in Ghee (Butter Fat) as Related to Structure of Triglycerides

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Granulation of buffalo and cow ghee (butter fat) as influenced by storage period and glyceride structure was studied. Buffalo ghee produced grains of larger size and were higher in quantity. The solid (grains) fraction of ghee differed from the liquid fraction and from whole ghee in physico-chemical characteristics and fatty acid composition. Distribution of fatty acids in triglycerides was found to be similar in buffalo and cow milk fats. Difference in the granulation behaviour of buffalo and cow ghee is discussed in relation to chemical composition and triglyceride structure.

Milk fat is a heterogeneous mixture of complex triglycerides. There is a direct relationship between chemical composition and physical properties of a fat[1]. Fatty acid composition was considered as the major factor that influences the physical behaviour of the fat. However, Desnuelle and Savary[2] demonstrated that positional distribution of fatty acids in the triglyceride molecule also determines the physical properties. Crystallization or granulation in ghee is one such property that might be influenced by fatty acid composition, glyceride structure etc. Completely melted ghee, on cooling to room temperature, can assume the form of large, coarse grains suspended discretely or in clusters in a liquid phase, and this phenomenon is considered by the common buyer as an important criterion of quality and purity. Though milk fat crystallization has been studied extensively[3-5], information on granulation of ghee is limited[6,7]. The present investigation is an attempt to correlate granulation of buffalo and cow ghee with differential make-up of their constituent triglycerides and fatty acids.

Materials and Methods

Pooled samples of milk were collected separately from buffaloes and cows of the Institute's herd. Ghee was prepared by the cream-butter method.

Granulation: Buffalo and cow ghee samples were melted at 80°C and 100 ml samples of each were kept at 29°C for 1-7 days. Granulation was compelte on the third day, and this temperature-time combination was used in all subsequent studies. The size of 100 granules was measured with a microscope with the help of an optical micrometer calibrated with a stage micrometer. Grains were separated by filtration through cheese cloth and the quantity of liquid fraction was recorded.

Physico-chemical constants: Ghee samples and their fractions were analysed for their physico-chemical characteristics like melting point, and Reichert, Polenske, Iodine and Saponification values according to Indian Standards Institution Methods[8].

Polyunsaturated fatty acids (PUFA) contents were measured by the alkali isomerisation method described by the AOCS[9].

Pattern of lipase hydrolysis: Susceptibility of whole ghee and of solid (grains) and liquid fractions to pancreatic lipase hydrolysis was determined by the method of Jack *et al*[10] with the exception that NH_4Cl-NH_4OH

buffer was replaced by 1 M NaCl as suggested by Freeman *et al*[11], in order that the progress of the reaction could be followed by titration with 0.1 N NaOH to maintain a constant pH of 8.0. A pH meter was used to monitor the pH changes during lipase action. Hydrolysis was carried out for a period of 20 min.

Intramolecular distribution of fatty acids in triglycerides: Buffalo and cow milk fats were extracted with chloroform-methanol (2:1) and triglycerides were isolated by preparative TLC described by Kuksis and Breckenridge[12]. The triglycerides thus obtained were hydrolysed for 3 min with pancreatic lipase (Sigma) by the method of Brochkerhoff[13]. The glycerides and fatty acids were then extracted with ethyl ether-hexane (1:1) from the reaction mixture. Monoglycerides obtained by lipase action were isolated by preparative TLC on 20 × 20 cm plates coated with a thin layer (0.5 mm) of silica gel G[14].

Gas liquid chromatography: Methyl esters of fatty acids prepared following deMan[15] were analysed in a gas chromatograph (F & M Model 609) equipped with a flame ionisation detector. Column was packed with Gas Chrom-P (100-120) coated with 10 percent EGSS-X. Analysis was carried out at temperatures programmed from 90° to 180°C. The injection and detector temperratures were 280°C and 250°C respectively. Nitrogen at 90 ml/min was used as carrier gas. Peak areas were measured by triangulation, and results expressed as mole percentages. Triglyceride types were calculated from pancreatic lipase hydrolysis data following the method of Vander Wal[16].

Results and Discussion

Effect of storage period on granulation: Results of storage of ghee samples at 29°C, as shown in Table 1, indicate total granulation in both buffalo and cow ghee samples by the third day, on which the minimum percentages of liquid fractions (59 per cent for buffalo and 80 per cent for cow) and maximum grain size (420 μm for buffalo ghee and 108 μm for cow ghee) were recorded. The grain size was observed to increase from the first to the third day in both buffalo and cow ghee; thereafter grain size decreased gradually in buffalo but not in cow ghee. Similar observations were recorded by Joshi and Vyas[7] in commercial buffalo ghee on storage.

TABLE 1. EFFECT OF STORAGE PERIOD ON GRANULATION AT 29°C

Storage period (days)	Buffalo		Cow	
	Grain size (μm)	Liquid* (%)	Grain size (μm)	Liquid* (%)
1	359	69	95	91
2	413	65	105	85
3	420	59	108	80
4	417	59	100	80
5	388	59	96	79
6	357	60	102	80
7	334	59	99	80

*Average of 4 samples.

Physico-chemical characteristics: Buffalo and cow ghee samples and their solid (grains) and liquid fractions obtained after storage for three days at 29°C were analysed for various physico chemical characteristics (Table 2). The average size of grain was 410 μm and 98 μm for buffalo and cow ghee respectively. Average percentage of the liquid fraction was 62 for buffalo ghee and 83 for cow ghee. Buffalo ghee grains occurred in clusters with irregular margins. Cow ghee grains, on the contrary, were discrete independent spherulites composed of fine divergent monocrystals radiating from the centre. Influence of chemical composition and glyceride structure on the kinetics of crystallization

TABLE 2. PHYSICO CHEMICAL CHARACTERISTICS OF WHOLE GHEE AND ITS FRACTIONS OBTAINED AFTER 3 DAYS OF GRANULATION AT 29°C

Physico-chemical characteristics	Buffalo			Cow		
	Whole ghee	Liquid fraction	Solid fraction	Whole ghee	Liquid fraction	Solid fraction
Melting point (°C)	33.7	25.9	37.2	31.5	24.5	36.3
Reichert value	33.5	36.9	28.9	28.0	29.6	21.3
Polenske value	1.3	1.4	1.3	1.5	1.6	1.5
Saponification value	227	229	219	221	224	216
Iodine value	27.1	29.6	24.1	32.2	33.9	27.6
Size of grain (μm)	410			98		
Vol. of liquid fraction (%)	62			83		

All values are average of 2 samples except the vol. of liquid fraction which is the av. value of 4 samples.

has been studied by various workers[1,3,17,18]. Grains larger in size and number, as in buffalo ghee, may be attributed to the differential make up of the triglycerides and fatty acid composition of buffalo milk fat (Tables 4, 5 and 6). Crystal size and structure are determined by the number of nuclei formed, temperature, supersaturation, chemical composition etc.[1] It has been reported that high melting triglycerides increase the crystal size and number[17–18]. Buffalo milk fat is reported to contain a greater amount of high melting triglycerides (8.7 per cent) as compared to cow milk fat (4.9 per cent)[19] and this may explain why buffalo ghee grains are larger in size and higher in quantity. Higher melting point (33.7°) and lower iodine value (27.1) for buffalo ghee as compared to cow's (31.5° and 32.2°, respectively) also correlate with greater content of saturated fatty acids and lower amount of unsaturated acids in buffalo ghee (Table 4). A marked difference in the physico-chemical characteristics between the solid (grains) and liquid fractions of buffalo and cow ghee was also observed (Table 2). Commercially feasible methods for separating milk fat by crystallization into high melting and low melting fractions with a wide range of physico-chemical properties have been developed by many workers[20-22]. Such fractionation processes provide a continuous economical method of modifying the physical properties of milk fat to make it more suitable for various purposes.

Polyunsaturated fatty acids (PUFA): All the PUFA from di-enoic to penta-enoic of conjugated and non-conjugated types were found to be at a higher level in the liquid fraction than in whole ghee or in the solid fraction (Table 3). Greater contents of PUFA and oleic acid in the liquid fraction were confirmed by higher iodine value. El-Sadek *et al*[23]. fractionally crystallized buffalo milk fat from acetone, and showed that conjugated and non-conjugated di-enoic and tetra-enoic acid contents in the liquid fractions increased as the temperature of crystallization decreased.

Pattern of lipase hydrolysis: Lipase hydrolysis data for buffalo ghee and its fractions are shown in Fig. 1. A similar pattern of hydrolysis for cow ghee and its fractions was also observed. The rate of hydrolysis of buffalo ghee was slower than that of cow ghee. A slower rate of hydrolysis for the solid fraction of each buffalo and cow ghee was observed as compared to the corresponding whole ghee and the liquid fraction. Lipase hydrolysis that takes place at an oil/water interface is

TABLE 3. POLYUNSATURATED FATTY ACIDS OF WHOLE GHEE AND OF LIQUID AND SOLID FRACTIONS OBTAINED AFTER 3 DAYS OF GRANULATION AT 29°C

	Conjugated (%)		Non-conjugated (%)	
	Buffalo	Cow	Buffalo	Cow
Di-enoic				
Whole ghee	0.7280	1.0010	0.6373	0.6168
Liquid fr.	0.7830	1.0374	0.6540	0.6432
Solid fr.	0.6550	0.8190	0.4354	0.4919
Tri-enoic				
Whole ghee	0.0540	0.0522	0.3431	0.4442
Liquid fr.	0.0570	0.0522	0.4795	0.4486
Solid fr.	0.0509	0.0451	0.3465	0.3441
Tetra-enoic				
Whole ghee	0.0085	0.0076	0.1517	0.1106
Liquid fr.	0.0090	0.0080	0.1582	0.1110
Solid fr.	0.0081	0.0060	0.1046	0.0962
Penta-enoic				
Whole ghee	0.0033	0.0037	0.0644	0.0764
Liquid fr.	0.0038	0.0041	0.0853	0.0809
Solid fr.	0.0031	0.0033	0.0535	0.0616
Total-PUFA				
Whole ghee	0.7938	1.0645	1.1965	1.2480
Liquid fr.	0.8528	1.1017	1.3770	1.2837
Solid fr.	0.7171	0.8734	0.9400	0.9938

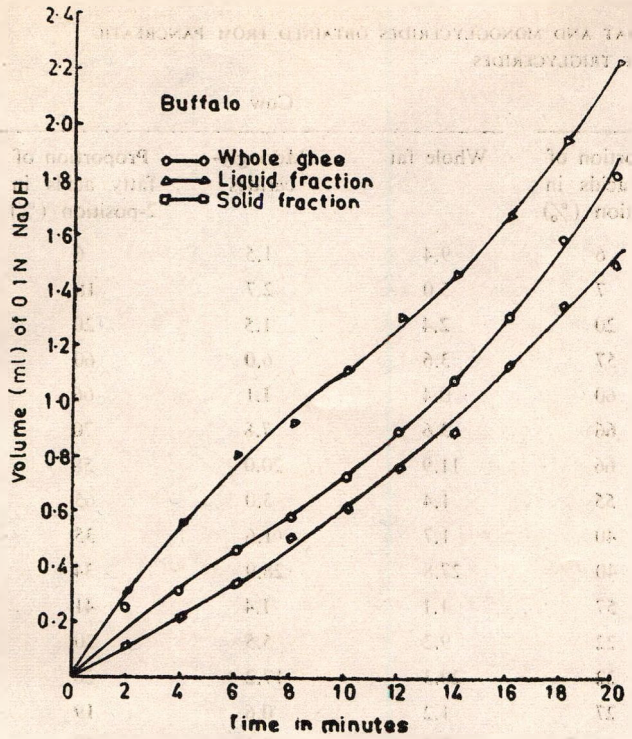

Fig 1. Pattern of lipase hydrolysis

affected by unsaturated fatty acids, type of triglycerides, physical state of the substrate, etc[24-25]. The lower rate of hydrolysis of the solid fraction may be due to the higher level of long-chain saturated fatty acids (Table 4).

Fatty acid composition: Table 5 shows the fatty acid composition of buffalo and cow ghee and of solid (grains) and liquid fractions. Higher contents of C_4, C_{16}, and C_{18} fatty acids and a lower level of $C_{18:1}$ fatty acid seem to be major points of difference between the fatty acid composition of buffalo and cow ghee. The percentages of long-chain saturated fatty acids, viz. C_{14}, C_{16} and C_{18} were higher in the solid fraction. Unsaturated and short chain fatty acids were concentrated in the liquid fraction. The fatty acid compositions of whole ghee and their fractions showed a direct relationship with their physico-chemical constants, (Table 2). Greater concentrations of C_{14}, C_{16} and C_{18} fatty acids in buffalo ghee can be correlated with grains larger in size and quantity as these fatty acids have been positively correlated with high-melting triglyceride content[25].

Intramolecular distribution of fatty acids and triglyceride structure: Buffalo and cow milk fat triglycerides were subjected to pancreatic lipase hydrolysis to study the distribution of fatty acids between primary and secondary hydroxyl groups of triglycerides (Table 5). Short chain fatty acids (C_4, C_6 and C_8) showed a specificity for the external positions, whereas medium chain fatty acids, (C_{10}, C_{12} and C_{14}) were preferentially attached to the internal position of triglycerides. Among the long chain fatty acids, C_{16} did not seem to show any preference but C_{18} fatty acids were found to concentrate in the external positions. The pattern of distribution of fatty acids between external and internal positions

TABLE 4. FATTY ACID COMPOSITION (MOLE %) OF WHOLE GHEE AND OF LIQUID AND SOLID FRACTIONS (FR.) OBTAINED AFTER 3 DAYS OF GRANULATION AT 29°C

Fatty acids	Buffalo			Cow		
	Whole ghee	Liquid fr.	Solid fr.	Whole ghee	Liquid fr.	Solid fr.
4:0	12.8	14.9	10.2	9.4	11.6	7.4
6:0	5.2	5.8	4.2	5.0	6.1	4.4
8:0	1.6	2.5	1.0	2.4	2.6	2.4
10:0	2.4	3.7	2.2	3.6	3.7	3.0
10:1	0.2	0.3	0.2	0.4	0.7	0.6
12:0	3.0	3.0	3.0	3.6	3.9	3.6
14:0	12.5	10.8	14.2	11.9	11.0	14.2
14:1	0.8	0.9	0.7	1.4	1.6	1.2
15:0	1.3	0.6	1.2	1.7	1.1	1.8
16:0	30.3	27.7	36.6	27.8	25.0	33.5
16:1	1.9	1.5	1.2	1.1	1.6	1.1
18:0	10.2	6.2	11.2	9.2	7.2	12.0
18:1	16.0	20.5	12.7	20.3	22.1	15.7
18:2	0.9	0.9	0.8	1.2	1.2	1.0
18:3	0.7	0.7	0.6	0.7	0.8	0.6

TABLE 5. FATTY ACID COMPOSITION (MOLE %) OF WHOLE FAT AND MONOGLYCERIDES OBTAINED FROM PANCREATIC LIPASE HYDROLYSIS OF TRIGLYCERIDES

Fatty acid	Buffalo			Cow		
	Whole fat	Monogly-cerides	Proportion of fatty acids in 2-position (%)	Whole fat	Monogly-cerides	Proportion of fatty acids in 2-position (%)
4:0	12.8	2.5	6	9.4	1.5	6
6:0	5.2	1.5	7	5.0	2.7	18
8:0	1.6	1.0	20	2.4	1.5	20
10:0	2.4	4.6	57	3.6	6.0	60
10:1	0.2	0.4	60	0.4	1.1	66
12:0	3.0	6.3	66	3.6	7.8	70
14:0	12.9	22.8	66	11.9	20.0	58
14:1	0.8	1.2	55	1.4	3.0	65
15:0	1.3	1.6	40	1.7	1.6	35
16:0	30.3	36.3	40	27.8	28.9	34
16:1	1.9	3.3	57	1.1	1.4	41
18:0	10.2	6.6	22	9.2	5.5	20
18:1	16.0	11.0	22	20.3	17.2	26
18:2	0.9	0.7	27	1.2	0.6	19
18:3	0.7	0.5	30	0.7	0.4	20

*Percentage of fatty acid esterified in the 2-position of triglycerides. Calculated from $M/3T \times 100$ where M = mole per cent of the acid in the monoglycerides, and T = mole per cent of the same acid in the triglycerides before hydrolysis.

of triglycerides seemed to be similar in both buffalo and cow milk fat triglycerides (Table 5). The difference in the physical behaviour of buffalo and cow milk fats may thus not be due to the pattern of distribution of fatty acids in the triglyceride molecules. However, Desnuelle and Savary[2] demonstrated how physical characteristics of natural fats are governed by the preferential distribution of fatty acids, in the light of data obtained by pancreatic lipase hydrolysis of the fats.

Triglyceride types calculated as described by Vander Wal[16] on the basis of pancreatic lipase hydrolysis data are presented in Table 6. A greater content of trisaturated glycerides (SSS) in buffalo milk fat (49.25 per cent) as compared to cow milk fat was found to be a major difference in the make up of the triglycerides of those two species. Ast and Vander Wal[26] and Coleman[27] calculated triglyceride types of various natural fats including cow milk fat and discussed the influence of triglyceride structure on properties of fat. It is tempting, therefore, to state that the higher level of trisaturated glycerides in buffalo milk may be one of the factors that contribute to the difference in the granulation of buffalo and cow ghee.

TABLE 6. TRIGLYCERIDE TYPES CALCULATED FROM PANCREATIC LIPASE HYDROLYSIS OF TRIGLYCERIDES

Triglyceride type	Buffalo (%)	Cow (%)
SSS	49.25	42.18
SSU	14.30	14.44
USS	14.30	14.44
SUS	10.81	13.32
USU	4.15	4.94
UUS	3.14	4.56
SUU	3.14	4.56
UUU	0.91	1.56

The present study indicates that the differential make-up of buffalo and cow milk fats in terms of fatty acid composition and triglyceride types may be a factor that controls granulation of ghee. Further investigation in this regard, like crystallization kinetics and a detailed investigation of triglyceride structure of buffalo milk may lead to a clear understanding of the difference in the physical behaviour of buffalo and cow milk fats.

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Processing of Teakseed *(Tectona grandis)* for Oil*

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Teakseed (*Tectona grandis*) has been processed for oil on a pilot plant scale. The seed is dehulled and the separated meats are extracted with normal hexane. The kernel content of teakseed is 2 percent. The kernel has 40 percent oil and 37 percent protein. The extracted kernel meal can be ground into a soft-textured, pinkish-coloured flour of good taste having 60 per cent protein and 4.5 percent crude fibre. Teakseed oil has an iodine value (Wijs) of 122, saponification value of 189 and unsaponifiable matter of 1.5 percent. Raw teakseed oil is deep red in colour but refined and bleached oil is yellow.

Teak, a verbenaceous tree (*Tectona grandis*) is indigenous to India, Burma and the Western parts of Thailand. It is also found in Java and some other Indonesian islands. Natural teak forests of India are confined mainly to the peninsular region. Under the current intensive campaign of forestry development, teak has been planted in the forests, parks and gardens of Uttar Pradesh (as far north as Dehra Dun), West Bengal, Assaam, Bihar, Orissa and the Andamans. While importance is being given to teak wood, no attention is being paid to the utilisation of teakseeds that are produced in large quantities in these forests. It is estimated that about 0.3 million tonnes of teakseed are available annually. In Karnataka State alone, the estimated quantity of seed is 75,000 tonnes. Teakseed is not utilized at present except for a very small quantity for regeneration purposes. An attempt has been made to process teakseeds for oil and meal. Teakseed oil has been reported by Puntambekar and Krishna[1] to contain palmitic, 6; stearic, 19; and oleic and linoleic acids, 75 percent; while Subrahmanyam and Achaya[2] reported myristic, 0.2; palmitic, 11; stearic, 10.2; arachidic, 2.3;

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TABLE 1. CHARACTERISTICS OF TEAKSEED

	Seed
Diameter, (mm)	11
Seed index g (100 seed)	56
Bulk density, (kg/M^3)	200
Calyx content (%)	8
Shell content (%)	90
Kernel content (%)	2
Appearance of the seed is irregularly round, buff to brown colour, hard and woody, covered in a loose sac like calyx.	

oleic, 29.5; linoleic, 46.4 and linolenic, 0.4 percent. The oil could therefore, constitute a good unsaturated oil for food use.

Materials and Methods

Materials: One metric ton of teakseed was procured from the Karnataka State Forest Department. The seed characteristics are given in Table 1.

Methods: Official and Tentative Methods of American Oil Chemists' Society (AOCS) were adopted for analysis of seed, oil and meal. The AOCS Combined Refining and Bleaching apparatus was used for refining of oil. Lovibond Tintometer was used to read oil colours.

The seeds were cleaned in a 3-deck mechanical seed cleaner fitted with suitable screens and dehulled in a disc huller. The mixture obtained was passed through a shaker-separator fitted with suitable screens (1.6 mm, 2.4 mm round holes and 3.2×6.4 mm slots) and air cyclone-separator. Yields of different fractions are given in Table 2. Kernels were analysed for proximate composition and the data are given in Table 3. The meat fraction obtained by dehulling the seed was extracted with n-hexane for recovery of oil. The physical and chemical characteristics of the oil are given in Table 4. The oil was neutralized with 16° Be' alkali lye and the neutralized oil was bleached with activated earth and activated carbon. Data on refinability of oil are given in Table 5. Solvent extracted deoiled meal was dried thoroughly and pulverized in a micro pulverizer. The proximate composition of teak kernel flour is given in Table 6.

TABLE 2. DEHULLING OF TEAKSEED

Fraction	Percent
Hulls (pure)	61
Powder (calyx and hulls)	28
Meats (kernels and hulls)	10
Processing losses	1

TABLE 3. COMPOSITION OF TEAKSEED KERNEL

Constituents	Percent
Moisture	5.1
Oil	40.0
Crude protein	37.0
Crude fibre	1.8
Total ash	5.0

TABLE 4. CHARACTERISTICS OF TEAKSEED OIL

Refractive index, (n_d 25°C)	1.4709
Specific gravity at 30°C	0.9145
Viscosity 32°C Redwood I, sec	177
Free fatty acids (%)	9.1
Iodine value (Wijs)	122
Saponification value	189
Unsaponifiable matter (%)	1.5

Discussion

Teakseed (actually fruit) also known as teaknut is hard and woody but light in weight and is enclosed in a loose sac-like calyx. Table 1 gives the characteristics and composition of the seed. The seed is 4-celled and contains two to four, mostly two or three soft kernels.

TABLE 5. REFINING CHARACTERISTICS OF TEAKSEED OIL

Free fatty acids of crude oil (%)	9	
Refining loss (%)	17	
Free fatty acids of refined and bleached oil (%)	0.2	
Colour in Lovibond Tintometer in0 .635 cm cell:		
	Yellow	Red
Crude oil	15.0	8.5
Refined oil	3.3	1.2
Bleached oil	2.4	1.0

TABLE 6. COMPOSITION OF TEAKSEED KERNEL FLOUR

Colour	Pinkish white
Moisture (%)	6.9
Oil (%)	2
Crude protein (%)	60
Crude fibre (%)	4.5

The kernels are ovoid in shape, and white in colour when fresh but turn pink on keeping. The kernel is very small in size and constitute only about 2 percent of the seed. This makes the dehulling and recovery of kernel from seed a difficult operation. During mechanical dehulling, the soft kernel was found smashed and lost in various fractions, with the result that only about half the total kernels present were obtained in the meats-rich fraction. The dehulled mixture was separated into three fractions, namely, pure hulls, hulls and calyx powder, and meats with some hulls (main fraction). The meats fraction shows a tenfold enrichment of kernel content over the original seed. Table 3 shows that the kernels contain 40 percent oil which is reasonbly high; however the oil content on original seed basis falls below 1 percent. Teak seed oil has saponification value of 189, but the literature value of 289.7 is high[3]. The iodine value is 122 whereas literature value is 105.8. The raw teakseed oil which is bright red is easily refinable to a light-coloured bland oil (Table 5). The extracted meal (flour) is soft, pinkish white and pleasantly sweet to taste and has a high protein content (Table 6) of 60 per cent.

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Chemical Composition of Pepper Grades and Products

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An attempt is being made to study the changes in chemical composition from the earliest maturity to the ripe stage and correlate this to the chemical composition of different pepper products and commercial grades. Piperine and volatile oil content increase up to just before full maturity. While starch increases with maturity, the crude fibre decreases. Analysis of various pepper products and maturity study indicated the optimum time for harvest of pepper.

The major constituents of pepper are starch, fibre and fat but more significant ones are the piperine and the volatile oil which contribute to pungency and aroma respectively. There are scattered reports about chemical analyses of various cultivars of black pepper in Kerala[1-4] as well as in different regions of India[5] and different trade grades and by-products[6]. Pruthi *et al*[7]. studied the optimum maturity for canned green pepper. Their results showed that in the earlier stages of maturity there was a drastic increase in starch and rapid lowering of volatile oil but little change was observed in non-volatile ether extract and piperine. Dwarakanath *et al*[2]. examined two varieties of pepper and found that concentration of starch increased in both cases, the results were conflicting in the content of crude fibre. The pattern of change observed in piperine content was not also very clear[2,8]. Lewis *et al*[9]. screened some important cultivars for preparing different pepper products.

Various factors of pepper and end uses in different forms are inter related[10]. In black pepper and oleoresin more value is attached to pungency followed by aroma. For preparing canned, bottled or dehydrated green pepper the raw material should have soft texture. For white paper, the raw material with mellowed flavour is preferred. Appearance, size, colour and density of the raw material also play a vital role. While plucking the berries to make black pepper the spike may contain berries at different stages of maturity. This may influence the size of the berry. A comprehensive study of the changes in chemical composition from the earliest maturity to ripe stage has not yet been done. This paper reports the

finding of such a study and the correlation of this to the chemical composition of different pepper products and commercial grades of pepper.

Materials and Methods

Pepper berries at different stages of maturity were sorted out from a single batch of harvest done at, Nedumangad area in Trivandrum District. "Mature stage" represented pepper of normal havest maturity. "Pin head stage" represented very small underdeveloped berries which normally dry to make up the grade pin heads. Between these two, three stages of maturity were identified as "between pin head and immature stage", "immature stage" and "under mature stage." Berries which turned yellow and red due to ripening were classified as "ripening stage". Pepper which have reached "under mature" and "immature stage" were green but hard and soft respectively by hand feeling. The different commercial grades of pepper as per Agmark grade specifications[11] were procured from an exporter's godown at Cochin. Light pepper consisted of berries which were hollow due to fungus and insect attack and were separated by air classification. Half pepper was slightly under matured berry with wrinkled surface whose density was between light and normal black pepper and which got separated during air classification. Buff coloured pepper was white pepper with skin and was prepared as per the process of Lewis *et al*[9].

Essential oil content was estimated by Clevenger distillation method[12], moisture by toluene distillation method; crude fibre, non volatile ether extract (NVEE) and starch by acid hydrolysis and Lane-Eynon procedure were determined by AOAC methods[13]. Piperine was estimated by spectrophotometric method[14]. Weight of a single berry was calculated by taking a fixed number of berries (20-100 depending upon the size) and dividing the weight by the number of berries.

Results and Discussion

The results of analysis of pepper berries of different maturities are presented in Table 1. Average weight of the berries increased up to about mature stage but during ripening the wet weight recorded a decrease. Moisture content was reduced from 82.5 per cent at pinhead stage to 60 per cent at mature stage and thereafter it dropped rapidly to about 40 per cent at the ripening stage. Because of this, the dry weight of berry continued to show an increase even at the ripening stage.

Concentration of volatile oil and piperine on dry weight basis, showed an increase up to just before full maturity and thereafter decreased up to ripening. Calculated on individual berry basis the volatile oil formation was quite fast up to just before maturation stage and thereafter there was no further formation. Some loss of volatile oil was observed during ripening probably coinciding with the sharp drip in moisture content. Accumulation of piperine in single berry reached maximum at about a stage slightly less than normal maturity and after that there was no further increase. A slight decrease observed during the last phase fo maturation and ripening may be due to sample variation.

Starch content increased during the entire maturation period. However, the increase was sharp during early maturation stages. Pruthi *et al*[7] reported that there was yet another increase during ripening stage. Contrary to Pruthi's[7] observation of a fairly constant value during ripening stages, the crude fibre content steadily dropped during maturation. This is despite the fact that on single berry basis the formation of fibre continues until the end. The decrease shown in the concentration is obviously due to large increase in dry weight contributed significantly by starch, which shows tremendous increase both on dry basis and single berry weight basis, and other constituents.

Data on the analysis of various commercial grades of pepper are shown in Table 2. Among the grades of increasing sizes like pin heads, Malabar and Tellicherry garbled, as expected, the weight of single berry increases. Both bulk density and density (volume by water displacement) also increased with increase in grade size.

TABLE 1. COMPOSITION OF PEPPER AT DIFFERENT MATURITY STAGES

Maturity stage	Moisture (%) (wet)	Av. berry wt.		Vol. oil		Piperine		Crude fibre		Starch	
		Wet (mg)	Dry (mg)	% (v/w)	Per berry (mg)	%	Per berry (mg)	%	Per berry (mg)	%	Per berry (mg)
Pin head	82.5	24.	4	2.0	0.080	0.4	0.016	18.0	0.720	15.3	0.612
Between pin head & immature	77.5	68	15	2.0	0.300	1.9	0.285	14.7	2.205	18.5	2.775
Immature	75.0	101	25	4.8	1.200	6.8	1.700	13.0	3.250	38.4	9.600
Undermature	65.0	151	53	4.4	2.332	6.2	3.286	11.8	6.254	38.4	20.352
Mature	60.0	156	62	3.7	2.294	4.2	2.604	10.5	6.510	40.9	25.358
Ripening	40.8	132	78	2.2	1.716	4.0	3.120	8.7	6.786	46.2	36.036

TABLE 2. ANALYSIS OF DIFFERENT GRADES OF PEPPER

Grades	Av. wt. of berry (g)	Bulk density (g/L)	Density (g/ml)	Moisture (%)	Vol. oil (%) (v/w)	Piperine (%)	NVEE (%)	Starch (%)	Crude fibre (%)
Pin heads	0.004	280	0.571	13.0	0.6	0.8	7.1	11.5	27.4
Light pepper	0.010	240	0.476	13.0	2.9	4.1	13.5	14.6	27.8
Malabar garbled 1	0.042	540	0.952	13.0	3.7	5.0	12.3	39.7	11.8
Tellicherry garbled extrabold	0.057	544	1.000	13.0	2.2	4.4	9.1	39.7	10.8
Tellicherry garbled special extra bold	0.061	592	1.053	10.0	3.2	4.9	10.3	40.9	9.2
Malabar ungarbled	0.039	540	0.952	12.0	2.8	5.0	11.4	41.8	12.5
Tellicherry ungarbled	0.034	528	0.952	12.0	4.0	6.3	13.5	39.3	11.0
High range ungarbled	0.040	600	1.053	12.0	2.6	4.0	11.1	41.8	10.5
Half pepper	—	—	—	—	4.2	6.8	13.1	—	—

Results are on dry wt. basis; NVEE: Non-volabile ether extract

Light pepper (as could be expected) shows a low bulk density and density, with low starch content and high fibre content. Mitra *et al*[15] have also noticed a lower starch content in light pepper and suggested that an estimation of starch can give an idea about the light pepper content. This grade consists of berries which are hollow inside due to microbial or insect attack. Since the core has most of the starch and less of fibre[16], it is understandable that light pepper shows a low starch and high fibre content. Pin head shows a low content of volatile oil, piperine and starch but high crude fibre content. Half pepper is believed to be of a lower maturity stage and it shows a high volatile oil and piperine content. This is a good raw material for oleoresin manufacture. With increasing grade size, while active constitutents like volatile oil and piperine do not show any definite pattern of change, the starch content by a small increasing trend and fibre content by a decreasing trend indicate the possibility of larger grades being of higher maturity stages. Among the ungarbled grades, Tellicherry ungarbled shows a higher volatile oil and peperine content. Probably this grade contains a larger proportion of berries of undermature or immature stage. Besides varietal changes, variations in the stage of maturity at the time of harvest can also influence the chemical constitutents.

Data on the analysis of different pepper products are given in Table 3. White pepper is usually made from mature samples and the product analysed reflects a high maturity. But in India, growers are unwilling to allow the pepper to ripen on the vines for fear of loss due to falling of berries and being eaten away by birds and hence the white pepper is made from black pepper. Because of this, the analytical data does not reflect a high maturity of pepper sample. Green pepper products on the other hand are prepared from immature pepper and therefore, piperine and volatile oil contents are quite high as evidenced from the products tested here.

To obtain the optimum concentration of the quality factors, it is desirable to harvest the pepper at the right maturity stage which will vary depending on its end use. Although at immature stage both piperine and volatile oil content sore higher, at the matrise stage for black pepper, the dry weight content is substantially higher and therefore, overall quantity of the active principles in a berry is not affected (Table 1). On the other hand for white pepper where mellow flavour is preferred, ripening stage will be ideal. Another advantage with white pepper is that in ground form it will not form detracts in white coloured soups and sauces. Because of maturation and removal of outer skin, the product will have a high starch and low fibre content and thus enable to form a very fine powder. In the buff coloured whole pepper, some of these advantages will be missing.

Since "half pepper" consists of immature berries, the grade will show high piperine and oil contents making it an ideal raw material for oleoresin extraction. Besides, due to the wrinkled surface, this grade is slightly

TABLE 3. ANALYSIS OF DIFFERENT PEPPER PRODUCTS

Sample	Piperine (%)	Vol. oil (%) (v/w)	NVEE (%)
Buff coloured pepper	5.1	1.8	9.7
White pepper	4.1	1.6	7.3
Dehydrated green pepper	8.0	3.8	11.8
Canned pepper	7.5	4.3	15.5
Bottled pepper	7.1	3.3	10.1

cheaper than normal black pepper. Between the garbled and ungarbled pepper, the latter will be better for extraction because of price advantage[17]. Similarly "light pepper" will be a good starting material for oil distillation because of cheaper price. Green pepper products because they are made from immature pepper will be usually high in piperine and oil concentration compared to black pepper.

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Extent of Phosphine Residues in Cereals and Cereal Products

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Wheat and maize grains were fumigated in metal containers with 2 and 4 tablets of aluminium phosphide per ton and the residues of phosphine were determined colourimetrically. The average residues immediately after exposure were 0.012 and 0.021 ppm in whole wheat grains and 0.015 and 0.031 pm in whole maize grains with 2 and 4 tablets respectively. The residues were below tolerance level of 0.01 ppm after 2 days of aeration. Preparation of flour and *chapaties* from the wheat immediately after exposure reduced the phosphine residues by 37 to 71 per cent and 90 to 96 per cent with respect to the deposit in whole grain. Similarly, the loss was 52 to 59 per cent and 93 per-cent in the preparation of *maize* flour and *chapaties* respectively. *Dalia* preparation resulted in loss of 28 to 39 per cent in case of wheat and 14 to 26 per cent in case of maize. Steam cooking of *dalia* completely reduced the residues except with the higher treatment dosage.

In India cereal grains are stored by agriculturists co-operatives and government[1]. Dewell[2] estimated that in India upto 10 million tonnes of foodgrains were lost each year due to faulty storage. Use of fumigants is recommended for safe storage of cereals. However, such fumigants may leave toxic residues in the grain which may prove hazardous to consumers. The absorbed toxic residues may also affect the acceptability and other qualities of cereals and their products. The presetn studies were therefore, aimed to study the extent fo phosphine residues in the fumigated wheat and maize and their processed products.

TABLE 1. RESIDUE OF PHOSPHINE IN WHEAT FLOUR AND *Chapati*

Dose (Tablet/ton)	Days after aeration	Whole grain		Flour			Chapati		
		Residue (ppm)	Reduction (%)	Residue (ppm)	Reduction (%) on the basis of		Residue (ppm)	Reduction (%) on the basis of	
					days	wholegrain		wholegrain	flour
2	0	0.012	—	0.003	—	71	0.0005	96	85
,,	2	0.004	65	BDL	100	100	BDL	100	100
,,	4	BDL	100	BDL	100	100	BDL	100	100
4	0	0.021	—	0.013	—	37	0.002	90	83
,,	2	0.011	48	0.003	70	6.3	BDL	100	100
,,	4	BDL	100	BDL	100	100	BDL	100	100

BDL—Below detectable level

Materials and Methods

Wheat and maize were filled in airtight galvanised iron sheet drums (diameter 62 cm and height 86 cm) and were fumigated by inserting "Quickphos" (aluminium phosphide) tablets wrapped in a wet cloth through the feeding hole and placed on the top of the cereal grains. In each drum, 140 kg of wheat or maize were filled and dosages of 2 and 4 tablets per ton were used. After inserting the tablets, the lid of the feeding hole was sealed with plastocin. An exposure period of 3 days was allowed.

Five hundred grams of the samples were collected at different time intervals. Since 3 days was the exposure period, the first sample was drawn 3 days after treatment and was designated as zero (0) day sample. The grain samples collected were processed immediately into *dalia* (cracked grain), flour and *chapatis* and analysed for residue content.

Phosphine content was determined by the colourimetric method of Bruce *et al*[3]. and as recommended by the Joint Committee of FAO/WHO on Pesticide Residues[4]. The recoveries of fumigant residues from the treated cereals after addition of known amounts of phosphine were worked out to be 76 to 81 per cent for wheat and 73 to 83 per cent for maize.

Results and Discussion

Whole wheat and maize grain: The deposits of phosphine (residues at 0-day of aeration) were 0.012 and 0.021 ppm in wheat (Table 1) and 0.015 and 0.031 ppm in maize (Table 2) in the lower and higher dosages respectively. The residues in wheat were reduced by 48-65 per cent after two days of aeration, whereas a greater reduction of 76 to 80 per cent was noted in maize at the same period. The residue levels of phosphine were below the detectable level 4 days after aeration.

Bruce *et al*[3] observed with 'phostoxin', a reduction from 0.046 to 0.006 ppm in wheat after aeration. Betraltiff *et al*[5]. reported that after 8 days there was no detectable residue in wheat sample. The phosphine residues

TABLE 2. REIDUES OF PHOSPHINE IN MAIZE, MAIZE FLOUR AND *Chapati*

Dose (Tablet/ton)	Days after aeration	Whole grain		Flour			*Chapati*		
		Residue (ppm)	Reduction (%)	Residue (ppm)	Reduction (%) on the basis of		Residue (ppm)	Reduction (%) on the basis of	
					days	wholegrain		wholegrain	flour
2	0	0.015	—	0.007	—	52	0.001	93	85
,,	2	0.003	76	BDL	100	100	BDL	100	100
,,	4	BDL	100	BDL	100	100	BDL	100	100
4	0	0.031	—	0.012	—	59	0.002	93	84
,,	2	0.006	80.3	0.002	84	66	BDL	100	100
,,	4	BDL	100	BDL	100	100	BDL	100	100

BDL—Below detectable level

TABLE 3. RESIDUES OF PHOSPHINE IN WHEAT *dalia* AND COOKED *dalia*

Dose (Tablet/ton)	Days after aeration	Whole grain		*Dalia*			Cooked *dalia*		
		Residue (ppm)	Reduction (%)	Residue (ppm)	Reduction (%) on the basis of		Residue (ppm)	Reduction (%) on the basis of	
					days	wholegrain		wholegrain	dalia
2	0	0.012	—	0.008	—	39	BDL	100	100
,,	2	0.004	65	0.0005	85	88	BDL	100	100
,,	4	BDL	100	BDL	100	100	BDL	100	100
4	0	0.021	—	0.015	—	28	0.001	95	92
,,	2	0.011	48	0.005	67	54	BDL	100	100
,,	4	BDL	100	BDL	100	100	BDL	100	100

BDL—Below detectable level

also reached below detectable level in about 1 to 2 days in wheat[6] and maize[7] and in 4 to 7 days of aeration in wheat[8].

The results also indicate that the residues of phoshine reached below the tolerance level of 0.1 ppm[9] in two days after aeration of wheat and maize fumigated with 2 and 4 tablets/ton.

Residues in wheat and maize flour and chapaties: Data in Table 1 and 2 indicate that when fumigated grains were converted into flour the average residue was found to be 0.003 and 0.013 ppm in wheat flour and 0.007 and 0.012 ppm in maize flour in case of 2 and 4 tablets of 'quickphos' per ton respectively. Residues from lower dosages in wheat and maize were below the tolerance limit, but not in the flours prepared out of grains treated with higher dosages. However, two days of aeration of the whole grain before conversion into flour reduced the residues by 63-66 per cent bringing it to the tolerance level of 0.01 ppm[9]. Since the whole grain did not contain residues after two days of aeration, the flours prepared out of them also showed no detectable residues. This suggests that wheat and maize flours would be fit for consumption if used after two days of aeration of the treated grain.

Dhaliwal and Lal[6] also reported that the flour of the fumigated wheat one day after aeration contained residues of 0.006, 0.008 and 0.013 ppm for the dosages of 2, 4 and 8 'phostoxin' tablest/ton, respectively.

Further, the *chapaties* made out of the flour of the treated aerated grain at zero-day showed residues ranging between 0.0005 and 0.002 ppm in wheat and 0.001 and 0.002 ppm in maize at the lower and higher dosages, respectively. The residues of phosphine in *chapaties* afterwards was below the detectable level. This low level of residue in *chapaties* (0.01 ppm) could be attributed firstly to the time lost in preparation of the *chapaties* and secondly the process of cooking. This suggests that preparation of flour from the treated grain and conversion to *chapaties* would pose no hazard to the consumer.

TABLE 4. RESIDUES OF PHOSPHINE IN MAIZE *dalia* AND COOKED *dalia*

Dose (Tablet/ton)	Days after aeration	Whole grain		*Dalia*			Cooked *dalia*		
		Residue (ppm)	Reduction (%)	Residue (ppm)	Reduction (%) on the basis of		Residue (ppm)	Reduction (%) on the basis of	
					days	wholegrain		wholegrain	dalia
2	0	0.015	—	0.012	—	14	BDL	100	100
,,	2	0.003	76	0.0005	90	87	BDL	100	100
,,	4	BDL	100	BDL	100	100	BDL	100	100
4	0	0.031	—	0.022	—	26	0.001	93	95
,,	2	0.006	80.3	0.003	86	50	BDL	100	100
,,	4	BDL	100	BDL	100	100	BDL	100	100

BDL—Below detectable level

Residues in dalia of wheat and maize: Data given in Tables 3 and 4 show that conversion of whole grain into *dalia* (cracked wheat and maize) reduced the level of phosphine residues. There was greater reduction in residues in wheat than in maize *dalia*. Maize *dalia* contained residues above tolerance limits of 0.01 ppm at zero-day of aeration which further declined to below the tolerance limit when prepared after two days of aeration. Wheat *dalia* also followed the same pattern. Cooking of *dalia* left no measurable residues except in the case of maize *dalia* prepared after zero-day of aeration.

The results on the phosphine residues in wheat and maize grains and their products show that aeration for two days after fumigation resulted in residues below permissible level, and hence would be fit for consumption. However, sensory evaluation pertaining to acceptability and organoleptic qualities is needed.

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Factors Affecting H_2S Level in Indian Wines

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Factors affecting H_2S content of wine prepared from varieties 'Early Muscat' and 'Beauty Seedless' have been examined. The major contributing factors are the variety of grape, and the temperature of fermentation which determine the amount of H_2S finally retained in the wines.

Hydrogen sulphide (H_2S), is one of the undesirable metabolites of yeast fermentation. Although, its importance in fermented beverages such as wine and beer have been recognised since long, few reports are available on the factors that contribute to the build up of this undesirable chemical in wines. A variety of factors such as the grape variety, the antiseptics used, the strain of yeast used, etc. have been suggested to be involved in the development of H_2S in wines[1-3]. In India, grapes used for wine production are mostly of table varieties, grown over a wide range of soil and agronomical practices. Also, fermentation conditions differ considerably from the traditional procedures used in wine producing countries. It was therefore, desired to initiate a study to examine the effect of various factors on the H_2S content of Indian wines. The availability of a technique described by Acree *et al*[4]. to measure total H_2S content in wines without significant interference from SO_3 facilitated quick and reliable measurement of the H_2S content of wines. This paper reports the contribution of a few factors on the H_2S content of wines prepared from the varieties of grapes locally used for wine production.

Materials and Methods

The yeast strain used in fermentation studies was *Saccharomyces cerevisiae* No. 522 which was originally from the Department of Enology, Univ. of California,

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Davis, U.S.A. and was maintained on YEPD-agar slants.

Two varieties of grapes used, namely 'Early Muscat' (White) and 'Beauty Seedless' (red) were from the experimental vineyard of this University. The grape juice from 'Early Muscat' and 'Beauty Seedless' had 17.0 and 17.2 per cent sugar, 3.4 and 3.3 pH and 1.0 and 1.2 g tartrate per 100 ml of grape juice, respectively. Grapes used for examining effect of Blitox on H_2S production were sprayed twice (at 16 and 5 weeks before harvest) with 0.2 per cent Blitox-50. During the spray operation, control plants were covered with polythene sheets.

Wine fermentation: Most procedures used were similar to that described by Amerine *et al*[5]. and unless otherwise stated, free run juice was ameliorated to 24° Brix with cane sugar. Potassium metabisulphite (KMS) was added at 150 ppm about 4 hr before inoculating the juice with a yeast culture previously grown in sterilized grape juice. Initial yeast cell density was maintained around 1.2×10^5 cells/ml. Fermentation was carried out at 15°C and whenever fermentation was carried out on the pomace, this was removed at a Brix reading of 10.

Measurement of total H_2S: Total H_2S in wine samples was determined after the first racking spectrophotometrically as described by Acree *et al*[4]. Quantitative determination of H_2S escaping in fermentation gases was made by passing in fermentation gases through a cadmium hydroxide trap and estimating the H_2S thus trapped as before. Two hundred ml of the juice from variety 'Early Muscat' was fermented in 10×2 in. glass tubes fitted with a rubber stopper carrying a bent tube dipping in a solution of 10 ml of alkaline cadmium hydroxide solution. The amount of H_2S trapped was determined at 3 hourly intervals using fresh solutions. The amount of total H_2S in wine is expressed in ppb after deducting the H_2S content of unfermented juice. All experiments were done at least in duplicate unless otherwise stated.

Results and Discussion

Effect of grape variety, pomace and KMS on H_2S content: The sulphur content of grapes has been reported to determine the H_2S content of wines[6,7]. Although this is mostly a varietal difference, the soil type and the agronomical practices also influence the final sulphur content of the varieties. To examine this, free run juice was extracted from the two varieties of grapes and about 700 ml of this was allowed to ferment in glass bottles (1 l.) after appropriate amelioration. After fermentation was complete, wines were racked and analysed for H_2S content. Wines prepared from free run juice without KMS from 'Early Muscat' contained slightly higher amount of H_2S than the wine from 'Beauty Seedless' (Fig. 1-A).

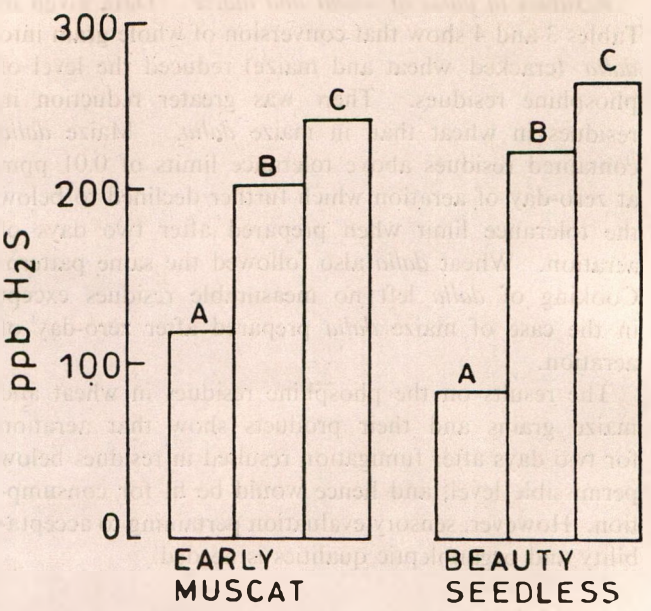

Fig. 1. Effect of fermentation conditions on H_2S content of wine.

A. Juice fermentation; B. Juice fermentation with KMS; C. Juice fermentation with Pomace and KMS

In wine fermentation KMS is generally used as an inhibitor for preventing the growth of undesirable yeast. To examine the extent to which KMS at 150 ppm contributes to the final H_2S content, free run juice was fermented after treating with KMS and analysed as above. It was found that wines from both varieties of grapes prepared by pretreatment with KMS contained nearly twice the amount of H_2S than wines fermented without, KMS. (Fig. 1-B).

For better flavour and colour extraction, fermentation is generally carried out on pomace. To determine the extent to which the pomace contributes to the H_2S content, free run juice fermented with pomace to 10° Brix and analysed at first racking. Wine from both varieties when prepared by fermentation on skin contained nearly $1\frac{1}{2}$ times more of H_2S than in wines prepared from only the free run juice. (Fig. 2) From these studies, it appears that the grape variety and the KMS contribute almost equally to the total H_2S content of the wine.

Fermentation temperature on H_2S content: The temperature at which fermentation is carried on varies with the type of wine to be prepared. For example, white wines are normally prepared at a lower temperature (15°C) with red wines at 25-30°C[5]. To examine the effect of temperature on the H_2S content of wines, fermetnation was carried out both at 15°C and 30°C and wines were analysed at first racking. It was found

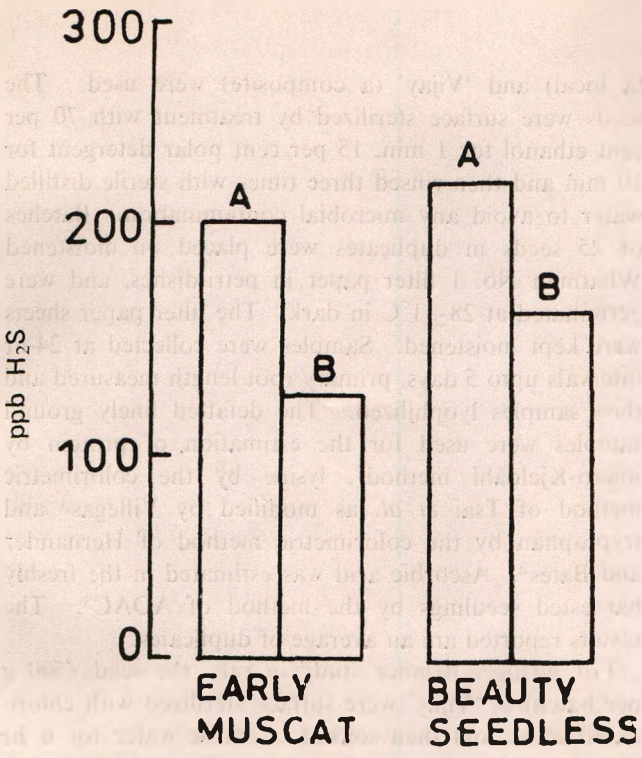

Fig. 2. Effect of fermentation temperature on H_2S content of wine

Wines were prepared using free run juice only.
A. 15 C; B. 30 C

that wines prepared from 'Early Muscat' and 'Beauty Seedless' had 60 per cent and 36 percent respectively more of H_2S when fermented at 15 C.

Blitox-50 spray and H_2S content: Some sulphur and non-sulphur fungitoxicants used in vineyards as plant protectants are reported to affect the H_2S level of wines[7,8]. Blitox-50, a non sulphur copper fungicide is generally used in this area as a plant protectant. To examine the residual effect of this fungicide on the H_2S level of wines, fermentations were carried out both with and without pomace and wines were analysed as before. It was found that wines prepared from both the varieties, with or without pomace, contained almost identical amounts of H_2S suggesting that Blitox-50 does not contribute significantly to the H_2S content of wines.

H_2S content of fermentation gases: The amount of H_2S left behind in wine represents that amount which remains after the escape of the excess gas during fermentation in the fermentation gases. To estimate the amount of H_2S produced during juice fermentation of 'Early Muscat', fermentation gases were passed through a cadmium hydroxide, trap. The amount of H_2S retained in wines was determined after the fermentation was completed. It was found that a total of 136.7 ppb of H_2S is lost in the gases during fermentation and an equal amount (113.7 ppb) is retained in the wine. Thus, a total of about 239 ppb (average of 4 replicates) of H_2S is produced during the fermentation of juice from variety 'Early Muscat'.

These studies are consistent with the earlier reports that a variety of factors determine the H_2S content of grape wines. The amount of H_2S produced during fermentation of both 'Early Muscat' and 'Beauty Seedless' is considerable. Major contributors to the final H_2S content of wines are the variety and the KMS used, however, the major agency bringing about this transformation is the yeast[8,9,10] since in unfermented juice the amount of H_2S is negligible (less than 10 ppb), In recent years, therefore, attempts have been made to select yeast strains that produce less of H_2S during fermentation. In a separate report, our work on the genetic control of H_2S production in yeasts and the derivation of yeast strains that excrete less amount of H_2S in wines will be reported.

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RESEARCH NOTES

IMPROVING NUTRITIVE VALUE OF MAIZE (*ZEA MAYS* L.) BY GERMINATION

Chemical and biological evaluation of nutririve quality of germinating seeds of two maize varieties—'Bassi' and 'Vijay' were carried out. Lysine and tryptophan contents increased substantially in both the varieties during germination upto 5 days. Increase in ascorbic acid content was also observed upto 3 days in 'Vijay' and upto 4 days in 'Bassi'. Rat feeding experiments indicated slight but distinct improvement in nutritive quality as seen by the improvement in biological value and utilizable protein percentage even at 30 hr imbibition (6 hr soaking + 24 hr germination) at 28°C.

The nutrtional quality of maize protein is poor because of very high proportion of zein (prolamin, 50-60 percent) which is extremely deficient in lysine and tryptophan but rich in leucine.

However, it has been reported[1] that during germination of maize, zein fraction is degraded and nutritionally superior and metabolically active proteins are synthesized. Therefore, the nutritional value of maize can be improved by the utilization of sprouted maize grain. In the present investigation a study of the changes in lysine, tryptophan, ascorbic acid and nutritional quality as determined by rat feeding experiment, in normal maize during germination has been undertaken.

Seeds of the two maize varieties namely, 'Bassi' (a local) and 'Vijay' (a composite) were used. The seeds were surface sterilized by treatment with 70 per cent ethanol for 1 min, 15 per cent polar detergent for 10 min and then rinsed three times with sterile distilled water to avoid any microbial contamination. Batches of 25 seeds in duplicates were placed on moistened Whatman No. 1 filter paper in petri-dishes, and were germinated at 28±1°C in dark. The filter paper sheets were kept moistened. Samples were collected at 24-hr intervals upto 5 days, primary root length measured and then samples lyophilized. The defatted finely ground samples were used for the estimation of protein by micro-Kjeldahl method[2], lysine by the colorimetric method of Tsai *et al.* as modified by Villegas[3] and tryptophan by the colorimetric method of Hernandez and Bates[4]. Ascorbic acid was estimated in the freshly harvested seedlings by the method of AOAC[5]. The results reported are an average of duplicates.

For nitrogen balance study in rats, the seeds (500 g per batch) of 'Vijay' were surface sterilized with chlorinated water and then soaked in sterile water for 6 hr before putting for germination. The samples were collected at 1, 2, 3 days, washed thoroughly with water and dried in air-oven at 50°C. The nitrogen balance for each sample was determined in five male albino rats, Wistar strain weighing about 70 g as described by Lodha *et al*[6]. Casein was used as control. Each rat received 10 g dry matter and 150 mg nitrogen daily.

In both chemical analysis and nitrogen-balance

TABLE 1. CHANGES IN PHYSICAL AND CHEMICAL CHARACTERISTICS IN GERMINATING MAIZE (ON PER KERNEL OR SEEDLING BASIS)

Maize variety	Time (days)	Primary root length (cm)	Dry wt (mg)	Protein (mg)	Lysine (μg)	Tryptophan (μg)	Ascorbic acid (μg)
Bassi	0	—	163	19.3	385	69	—
	1	0.0	162	19.7	385	71	0
	2	1.4	155	19.5	419	80	15
	3	4.6	155	19.5	626	111	30
	4	8.3	139	18.5	760	147	43
	5	Tips decayed	127	17.0	894	156	21
Vijay	0	—	275	29.8	789	113	—
	1	0.0	270	29.9	800	119	0
	2	3.3	266	29.6	832	141	9
	3	4.8	255	28.3	1005	160	28
	4	5.4	245	28.1	1102	193	21
	5	6.5	238	27.4	1197	214	9
Shakti Opaque-2	0	—	—	20.4	1029	174	—
Rattan Opaque-2	0	—	—	25.6	1155	176	—

TABLE 2. CHANGES IN PROTEIN, LYSINE, TRYPTOPHAN AND ASCORBIC ACID DURING GERMINATION OF MAIZE (ON PER CENT BASIS)

Maize Variety	Time (days)	Total protein (%)	Lysine (%)	Try-ptophan (%)	Ascorbic acid (mg/100 g)
Bassi	0	12.27	1.99	0.36	—
	1	12.50	1.96	0.36	0.0
	2	12.53	2.15	0.41	8.6
	3	12.56	3.21	0.57	17.4
	4	13.33	4.11	0.79	21.2
	5	13.35	5.26	0.92	15.3
Vijay	0	10.88	2.65	0.38	—
	1	11.06	2.68	0.40	0.0
	2	11.38	2.81	0.48	3.3
	3	11.00	3.55	0.57	8.5
	4	11.56	3.92	0.69	8.8
	5	12.29	4.37	0.78	3.7
Shakti Opaque-2	0	10.69	5.04	0.85	—
Rattan Opaque-2	0	11.47	4.51	0.71	—

studies, the germinated and non-germinated seeds were not separated at each stage but were dried and analysed together.

The dry weight per seedling decreased in both the varieties. However, the decrease was more in' Bassi' (22 per cent) compared to 'Vijay' composite (13 per cent) over a 5-days period of germination. The primary root growth was more in 'Vijay' as compared to 'Bassi' initially, but was comparable on 3rd day and was slower thereafter. In 'Bassi' the primary root growth occurred only upto 4 days and thereafter its tip started decaying, while in 'Vijay' the growth continued (Table 1).

The changes in protein, lysine, tryptophan and ascorbic acid per seedling during germination in 'Vijay' and 'Bassi' are given in Table 1. During germination the protein decreased by 12 per cent in 'Bassi' and 8 per cent in 'Vijay' while the level of lysine and tryptophan increased markedly. The increase in lysine was 2.3 and 1.5 fold, while in tryptophan it was 2.3 and 1.9 fold respectively in 'Bassi' and 'Vijay' during 5-day germination period. Ascorbic acid level per seedling also increased upto 3 days in 'Vijay' and upto 4 days in 'Bassi', and decreased thereafter in both the varieties.

Changes in protein and ascorbic acid contents on unit weight basis, in lysine and tryptophan as per cent of protein during germination are shown in Table 2. Protein content increased by 1.08 per cent in 'Bassi'and 1.41 per cent in 'Vijay' after 5 days germination. Lysine and tryptophan as per cent of protein also increased in both the varieties during this period. The increase in lysine and tryptophan content was respectively 2.6 and 2.5 fold in 'Bassi' and 1.6 and 2 fold in 'Vijay'. After 5 days germination, lysine content in 'Vijay' normal (4.37 per cent) became comparable to that of non-germinated opaque-2 counterpart 'Rattan' (4.51 per cent). Lysine in 'Bassi' normal (5.26 per cent) after 5 days germination was more than that of non-germinated 'Shakti' 'Opaque-2' seeds (5.04 per cent). Similarly, the tryptophan content of germinated seeds of 'Vijay' and 'Bassi' was slightly higher than that of 'Opaque-2' maize 'Rattan'' and 'Shakti'. During germination, ascorbic acid content increased to 21.2 mg/100 g in 'Bassi' and to 8.8 mg/100 g in 'Vijay' after 4 days, thereafter it showed a considerable decrease.

For biological value evaluation, 1, 2- and 3-days germinated seeds of 'Vijay' were used. The results indicating true digestibility (TD), biological value (BV), net protein utilization (NPU) and utilizable protein

TABLE 3. MEAN TRUE DIGESTIBILITY (TD), BIOLOGICAL VALUE (BV) AND UTILIZABLE PROTEIN (UP) OF GERMINATED SEEDS OF 'VIJAY' MAIZE

Protein value (%)	Non-germinated	Germinated*		
		1-day	2-day	3-day
TD	92.1 ±0.6	91.2 ±1.3	90.9 ±0.8	90.7 ±0.4
BV	56.4 ±2.9	65.2 ±1.5	63.8 ±2.6	65.9 ±4.2
NPU	52.2 ±2.5	59.5 ±1.9	58.0 ±3.4	59.8 ±3.8
UP	5.48±0.26	6.25±0.19	5.95±0.37	6.21±0.43
Protein in % of dry matter	10.50	10.50	10.25	10.38
Lysine (%)	2.82	2.99	3.21	3.63
Tryptophan (%)	0.39	0.44	0.50	0.55

*The seeds were soaked in water for 6 hr before putting for germination.

(UP) percentages are shown in Table 3. TD values of germinated 'Vijay' seeds were slightly lower than that of non-germinated seeds. However, the differences were not significant. In contrast to this there was a slight but distinct improvement in the BV of germinated seeds compared to non-germinated. BV even after one day germination improved by 15.6 per cent. Further increase in germination period did not result in increase in BV value. NPU value was also increased by 14 per cent after 1 day germination. The utilizable protein

$$\left(UP = \frac{NPU \times \text{Protein per cent}).}{100}\right)$$

also showed 14 per cent improvement at 1 day germination. The improvement in UP value is mainly due to the improvement in BV during germination as no significant change occurred in the protein content.

Recently, Chibber *et al*[7]. and Wang and Fields[8] have shown increase in lysine and tryptophan during germination in maize. However, the increase in the total lysine does not give true index of biological availability. In the present study also increase in lysine, tryptophan and Vitamin C contents has been observed during germination in two maize varieties. Inspite of little increase in lysine and tryptophan during early germination, distinct improvement in BV as well as UP has been observed by rat feeding experiment. This is for the first time that an improvement in BV on short germination period has been shown. The improvement in BV observed at 1-day germination could perhaps be due to the better availability of lysine and tryptophan from storage proteins. Longer germination periods would not be of much advantage as it would result in acceptability problems because of high crude fibre content due to vigorous root and shoot growth. The results thus indicate slight but distinct improvement in the nutritional quality of maize even at 30 hr imbibition. This should help in improving the nutritive quality of diets of people subsisting on maize.

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SOLUBILITY OF LACTOSE AND LACTULOSE IN ALCOHOLS

The degree of solubility of lactose and lactulose in methanol, ethanol and propanol was determined. The solubility decreased as chain length of the alcohol increased. Lactose has lower solubility in alcohols than lactulose. Purification of lactulose from mixtures with lactose can be achieved by extraction with alcohols. Optimum results were obtained using 95% methanol or 98% ethanol.

Lactulose (4-0-β-D-galactopyransoyl-D-fructose) is obtained from lactose by isomerization in basic media[1,2]. The potential interest of lactulose as an infant formula ingredient and as a therapeutic drug, has necessitated lactulose preparation from lactose. Following the first reported method for lactulose preparation[1], several other procedures have been published[3], all based on the principle that basic media provide the conditions for lactose rearrangement. However, the lengthy procedures involved in the purification of lactulose from the reaction mixtures, have limited the use of lactulose[4]. The differences in solubility of lactose and lactulose in alcohols can be used to separate lactulose from lactose. Although some work has been done on lactose solubility in several alcohol-water mixtures[5], no data are so far available on the solubility properties of either lactose or lactulose in different alcohols.

The solubility was measured after 30 min reflexing of an excess sugar in the corresponding alcohol. Samples were then kept in a water bath at 25°C for equilibration. After 24 hr all samples were filtered, and 10 ml of clear filtrate were dried (90°C for 24 hr) on a small aluminium dish and then weighed. In the case of lactose in ethanol and propanol, due to the low solubility, 150 ml of clear filtrate were taken to weigh the solids obtained after vaccum evaporation and heat drying as described above. The ratio of lactulose to lactose was determined by gas-liquid chromatography.

Dried samples were dissolved in trimethylsilylimidazol in pyridine (Tri-Sil-Z) and injected a few minutes later. Separation was carried out on a Perkin-Elmer

TABLE 1. SOLUBILITY OF LACTOSE AND LACTULOSE IN ALCOHOLS (G/100ML AT 25°C)

(%) water	Methanol		Ethanol		n-Propanol	
	Lactose	Lactulose	Lactose	Lactulose	Lactose	Lactulose
0.1	0.1312	1.980	0.0215	0.1643	0.0088	0.1042
2.0	0.1644	3.773	0.0285	0.2723	0.0094	0.1702
5.0	0.2953	4.960	0.0456	0.8202	0.0190	0.1864

TABLE 2. PURIFICATION OF LACTULOSE FROM EQUIMOLAR MIXTURE WITH LACTOSE

Solvent	Solubility (g/100 ml at (25°C)	Soluble fraction composition	
		% lactose	% lactulose
Methanol (99.8%)	2.2130	10.3	89.6
Methanol (98%)	4.6210	17.7	82.2
Methanol (95%)	5.4620	8.5	91.4
Ethanol (99.8%)	0.2483	20.5	79.4
Ethanol (98%)	0.3661	7.9	92.0
Ethanol (95%)	1.3240	16.9	83.0

model F-30 with stainless steel column 3m × 3mm packed with 3 per cent OV-17 on Chromosorb W-HP 80-100 mesh, 230°C column temperature, 285°C injector temperature, and N_2 as carrier gas at 25 ml/min. Proportional amounts of each compound were calculated by triangulation. All trimethylsilylated samples were analyzed in duplicate, differences between them being below 0.6 per cent.

Table 1 shows that lactose and lactulose solubility decreases with the increase in the chain length of the alcohol. The presence of water in alcohols raises the solubility of both sugars. Solubility of lactulose in alcohols is higher than that of lactose. Purification of lactulose from equimolar mixture with lactose was carried out by refluxing the mixture with the corresponding alcohol and filtering after samples were stored at 25°C for 24 hr.

Table 2 shows the composition of the sugar fractions obtained, alter extraction with methanol and ethanol.

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IDENTIFICATION OF THE MACE PIGMENT

The red pigment of mace was identified to be lycopene by TLC and absorption studies.

The colour of mace is an important factor influencing its commercial value. Mace which is the dried aril of the nutmeg (*Myristica fragrans* Houtt) has an attractive scarlet colour at the time of harvest. Usually it is shade dried to get the colour retained to the maximum level. On drying and storage, the colour gradually fades to yellow. Studies done so far[1-3] gives no information on the chemical nature of the colour present in it. An appropriate postharvest technology for mace necessarily requires an understanding of the chemical nature of the colour pigment for which purpose the present study was initiated.

Raw mace separated from freshly harvested nutmeg from Trivandrum area was used for the study. The colour was extracted in acetone and a little extract was shaken with light petroleum followed by dilution of the acetone with water. The whole colour was transferred to the petroleum layer thereby indicating its nonpolar and lipophilic nature like that of carotenes and absence of polar colouring matters like anthocyanins. It also gave characteristic blue colour with concentrated sulphuric acid as in the case of carotenes. The acetone extract was saponified to remove any lipid material present and extracted in petroleum ether. The petroleum extract was tested for the presence of xanthophylls, if any, by phase separation with 90 percent methanol followed by 95 per cent methanol as per standard procedure[4], but they were found to be absent. The red colour was concentrated and purified by column chromatography on alumina using petroleum ether as solvent.

TABLE 1. VISIBLE ABSORPTION MAXIMA OF MACE PIGMENT AND LYCOPENE ISOLATED FROM TOMATO

Pigment	Absorption maxima (n.m.) in different solvents		
	Light petroleum	Chloroform	Carbon disulphide
Mace pigment	445, 471, 501	456, 483, 517	477, 505, 545
Lycopene from tomato Observed value	445, 471, 501	456, 483, 517	477, 505, 545
Reported value[4]	446, 472, 505	456, 485, 520	477, 507.5, 548

$E^{1\%}_{1\,cm.}$ at 471 nm was found to be 3280 in hexane as against a reported value of 3450 for lycopene

The pigment gave absorption maxima at 445, 471 and 501 nm in light petroleum (60-80°C). It showed close resemblence to lycopene. Hence pure lycopene was isolated from tomato (*Lycopersicon esculentum* Mill) by standard procedure for carotenes as in the previous case. On TLC analysis on silica gel G plate of 1 mm thickness using (*a*) light petroleum (60-80°C)/benzene (98:2) and (*b*) hexane/acetone (95:5) the mace colour gave only one spot which was found identical with lycopene isolated from tomato (Rf value 0.19 and 0.90 respectively). The absorption maxima of the pigment in three different solvents were also found identical with those of reference sample and reported values for lycopene (Table 1). The IR spectra of the mace colour was also found to be identical with that of lycopene isolated from tomato which suggest the colour in mace is lycopene. As in the case of lycopene the mace pigment separated in dry form had extremely low stability and was very much light sensitive.

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INHERENT ACIDITY OF SOME TOMATO VARIETIES IN RELATION TO THEIR SHAPE

The relationship between fruit shape and acidity was investigated using twenty varieties of tomatoes (13 yielding round fruits and 7 yielding oblong fruits). Acid content among all the varieties ranged from 0.360 to 0.715 per cent The acidity level was more in round tomato varieties as compared to oblong varieties. The finding has been substantiated by the statistical analysis of data.

Shape and size of the fruits of tomato cultivars grown in this country vary widely. On the basis of fruit shape, these cultivars could be broadly divided into two, groups viz. those round in shape and others oblong. The inherent acidity of tomato has peen reported to be dependent on factors like variety, fruit maturity, fertilizer application and agro-climatic conditions[1]. Substantial differences in the acidity between different varieties grown at some locations have been reported by Bradley[1]. Variation in acidity within the same variety grown at different locations, due to seasonal and climatic conditions as well as cultural practices has also been reported by Bradley[1] and Saimbhi[2]. Decrease in acidity during maturation has been reported by Villarreal *et al*[3]. and the increase during ripening of the fruits was noticed by Belucci *et al*[4]. Relationship between the shape of the fruit and inherent acidity is discussed in this note.

Twenty tomato varieties (13 round fruit type and 7 oblong fruit type) grown at Experimental Farm, Hessaraghatta of the Indian Institute of Horticultural Research, Bangalore, were used for the present study. These varieties were grown in randomized block design with two replications keeping cultural practices same for all. Fruits at the same maturity stage (when they were full red in colour) were harvested from each replication and analysed separately. Acidity in the juice was determined by the A.O.A.C. method.[5] The acidity is expressed as per cent anhydrous ctiric acid. The average values calculated from two replications are reported in Table 1.

The acidity of different varieties of fruits analysed ranged from 0.360 to 0.715 per cent. Variety '598 Bulk' having lowest acidity of 0.36 per cent had oblong fruits, while 'Pusa Ruby' cultivar with round fruit had highest acidity of 0.715 per cent. The acidity of juice ranged from 0.420 to 0.715 per cent in the case of varieties having round fruits and from 0.360 to 0.450 per cent in the case of oblong varieties.

Differences in acidity among oblong varieties were not

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TABLE 1. INHERENT ACIDITY OF SOME TOMATO VARIETIES IN RELATION TO THEIR SHAPE

Variety	Fruit shape	Acidity (as anhydrous citric acid %) (W/V)
Selection — 1	Round	0.420
,, 2	,,	0.435
,, 3	,,	0.475
,, 6	,,	0.465
,, 7	,,	0.525
,, 13	,,	0.495
,, 16	,,	0.515
,, 24	,,	0.425
Pusa" Ruby	,,	0.715
Pioneer	,,	0.445
Khebras	,,	0.450
Ogesta	,,	0.575
602-1-SB	,,	0.605
Selection —11	Oblong	0.435
,, 17	,,	0.420
,, 18	,,	0.390
,, 25	,,	0.440
598 Bulk	,,	0.360
Punjab Chhuhara	,,	0.450
Gamad	,,	0.425

GM for round = 0.5034 GM for oblong = 0.4171

statistically significant but significant in the case of round varieties. The acidity level was more in round tomato varieties as compared to oblong varieties and the difference was statistically significant.

In general, oblong fruits have lower inherent acidity as compared to round fruits. Thus fruit shape and juice-acidity relationship may be helpful in the selection of right type of tomatoes for processing. During these studies, it was also observed that oblong varieties had a better keeping quality with lesser spoilage and longer shelf life as compared to round varieties. It will, therefore, be worth studying the role of inherent acid in the post-harvest physiology of the fruits.

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STATUS REPORT

Development of Food Information Services in India

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Food Science and Technology Information Service (FOSTIS), at CFTRI, Mysore has been approved as the National Information Centre for Food Science and Technology (NICFOS) under the NISSAT. This paper considers the need for the national information centre and describes the planning of the information centre and the various steps taken to implement the plan. The details of various services of the information centre are given.

Introduction

One of the country's main problems is to meet the food needs of its growing population. Our successive plans have rightly made substantial investments towards food and agriculture and, as a result, the agricultural production has almost doubled since in dependence. However, these efforts would become wasteful if parallel efforts are not made to reduce the post harvest losses of food grains that occur in the field of storage. Application of modern food technology is therefore, essential to minimise the losses as also to produce new and nutritious processed foods which can facilitate better utilisation of available food materials.

The establishment of CFTRI in 1950 is a major step in resolving this problem. This institute has since emerged as an R & D and training institute of international standing. During the last decade and a half, many universities and training institutions in India have started courses in food science and technology. There has also been a significant increase in the number of food industries also.

All the activities in food research and the industrial exploitation of research results need to be sustained and improved upon if the country has to reach self reliance in foods. One of the important requirements in this direction is an efficient information service which can effectively serve food scientists and technologists, consultancy and extension workers, planners and administrators, and the managerial and technical personnel of the food industries. Good infrastructure for such a service exists in CFTRI and in various other training and R & D organisations as well as a few prominent industrial units. The CFTRI being the premier institution in this area took the initiative and prepared a plan for a national information centre. Food Science and Technology Information Service (FOSTIS), has now been approved as the National Information Centre in Food Science and Technology (NICFOS) under the National Information System for Science and Technology (NISSAT) plan, of the Department o f Science and Technology, Govt. of India.

This report deals with the various facets in planning and establishment of NICFOS.

Publications in Food Science and Technology

In 1966, it was estimated that about 16,500 scientific papers were being published in about 1100 periodicals[2]. About 87.3 per cent of this were covered by about 460 periodicals. This however, does not include books, technical reports, patents, standards etc. Of the 90,000 abstracts included in the first 6 volumes of Food Science and Technology Abstracts (FSTA) published by the International Food Information Services (IFIS), UK, about 1000 were book reviews and 2000 were patents[3]. Reports and Standards would be about 1000. Further, when FSTA started publication in 1969 based on a survey, it included only 717 periodicals which increased to 1200 in 1977. Based on these, the current annual publication of food literature can be estimated at about 35,000.

The various disciplines of Food Science and Technology are interconnected and the information service should take cogniscence of this before planning for an organised information system. An analysis of the various disciplines and their interconnections are shown in the modified chart of Borgstrom[4] (Fig. 1).

The Indian Situation

In India, with the developments in food research, considerable information is being generated and published by a variety of institutions. While there are at least 100 Indian periodicals publishing information in food science and technology, only about 50 are being covered in FSTA at present. This emphasises the need for an Indian agency to procure all the Indian information and cater to Indian requirements and to feed the international systems to improve their coverage.

There are several research and teaching institutions

PERIPHERAL AREAS

BIOLOGICAL SCIENCES	MEDICAL SCIENCES	CHEMICAL SCIENCES	PHYSICAL SCIENCES	MATHEMATICAL SCIENCES
Biology	Physiology	Radio Chemistry	Physics	Mathematics
Radiobiology	Nutrition	General chemistry	Radio physics	Statistics
Zoology	Metabolism	Organic chemistry	Thermodynamics	Operations research
Parasitology	Nutritional disease	Physical chemistry	Cryophysics	Systems analysis
Entomology	Metabolic disease	Analytical chemistry		Computer science
Botany	Dietetics	Synthetic chemistry		
Microbiology	Diet therapy	Biochemistry		
Bacteriology	Pharmacology	Molecular biology		
Mycology				

PERIPHERAL AREAS

AGRICULTURAL SCIENCES
- Agriculture
- Animal husbandry
- Fisheries
- Economic botany
- Plant nutrition
- Animal nutrition

ENVIRONMENTAL SCIENCES
- Surface sciences
- Material sciences
- Ocean sciences
- Atmosphere sciences
- Space sciences
- Defence sciences

CORE SUBJECTS — FOOD SCI. & FOOD TECH.

- Physico-chemical properties
- Sensory evaluation
- Colour
- Rheology
- Flavour
- Shelf life
- Food nomenclature
- Food composition
- Food chemistry
- Food Analysis
- Biochemistry of foods
- Food Microbiology
- Nutrients in foods
- Food toxicology
- Food spoilage
- Food additives
- Pesticides
- Residues
- Food adulteration
- Quality control

CORE SUBJECTS

- Food engineering
- Food machinery
- Plant design
- Instrumentation
- Process control
- Sanitation
- Food processing
- Milling
- Heat processing
  - Canning
  - Pasteurisation
- Dehydration
  - Air drying
  - Drum drying
  - Freeze drying
  - Foam-mat drying
- Low temperature storage
  - Refrigeration
  - Freezing
- Traditional methods
  - Curing
  - Smoking
  - Salting
  - Pickilng
- Irradiation
- Chemical preservation
- Packaging
- Storage
- Infestation control
- Handling
- Transport

ENGINEERING SCIENCES
- Civil engineering
- Mechanical engineering
- Electronic engineering
- Sanitary engineering
- Refrigeration engineering
- Packaging engineering
- Chemical engineering
- Chemical technology
- Bioengineering
- Biotechnology

USEFUL ARTS
- Home science
- Food craft

PERIPHERAL AREAS

Planning	Economics	Food industries	Food statistics	Sociological aspects
Management	Food economics	Food marketing	Food legislation	Dietary surveys
Futurology	Agricultural economics	Food distribution	Food inspection	Extension services
Food history				Consultancy services
				Mass communication

PERIPHERAL AREAS

Fig. 1. Food Science and Technology: A structural map

(Universities, Institutes of Technology and Agricultural Universities) conducting R & D work in the area of food in India. It has been estimated that currently in India there are over 10,000 food industries[5] of all sizes and kinds. It is evident that these organisations will be requiring different kinds of information which should be provided by a competent organisation. The information resources, services and needs of the the country was assessed by a questionnaire survey.[6] Out of the 316 organisations which replied the questionnarie 210 organisations (about 68 per cent) pointed out the inadequate access to food information. A larger number (86 per cent), however, desired to have an agency at the national level to fulfil their information requirements.

National Information Centre for Food Science and Technology (NICFOS)

Planning: A draft (national food information) plan was prepared[1] for establishing an information centre (initially called FOSTIS Plan) which would fulfill the country's information requirement. The objectives of the plan were in confirmity with the nation's overall plans for the development of food industry and of R & D in food science and technology. The plan also took an integrated view of the related and coordinate systems and sub-systems of the NICFOS as this would help in improved productivity in the services by ensuring maximum benefit from the available resources.

NICFOS net work: The plan found it helpful to organise the total NICFOS as a central facility in the CFTRI, Mysore and several separate Regional facilities at the CFTRI Experiment Stations located in Ludhiana, Lucknow, Nagpur, Bombay, Hyderabad and Mangalore. However, this decentralisation is limited only to solve the regional problems. The central facility would have centralised functioning such as procurement, technical processing etc., besides being the coordinating agency for the entire NICFOS net work to avoid wasteful duplication and uneconomic organisation. The libraries in establishements specialising in food science and technology in the country will serve as local information units.

NICFOS as part of NISSAT

The Science and Technology Plan which was included in the Fith Five Year Plan provided for a national information net work in the country (NISSAT) which included an information centre for food science and technology at the sector level[7]. The FOSTIS plan was adopted for this purpose.

Objectives: The NICFOS has the following objectives:

1. Provide documentation information service to the CFTRI, Mysore, the food industry and other organisations/individuals engaged in research, development and / or extension activities in food science and technology;
2. to function as a clearing house for information on all aspects of food science and technology; and
3. to collaborate in the integrated development of various documentation/information systems in food science and technology and related systems in the country as a whole and function as a feeder to similar international systems.

Functional organisation: The central facility of NICFOS is organised into (*i*) document acquisition and processing; (*ii*) documentation service; (*iii*) promotion of information use in industry etc.; (*iv*) reprography, printing and publication; and (*v*) secretarist.

The organisation chart of NICFOS is shown in Fig. 2.

Plan Implementation

Surveys: A survey conducted in respect of the above factors is mentioned earlier. A user evaluation survey for the current NICFOS services has also been conducted[8]. Another survey to elicit proposed users' opinion for computerised information service has also been completed. These surveys by providing valuable information on user requirements have given guidelines for proper orientation of NICFOS services.

Information resources development: The basic requirement for providing efficient information services is a strong information base. The existing document collection of NICFOS is therefore, being augmented to fill up the gaps and by new acquisitions. A directory of on-going projects in food science and technology in India has been compiled to bring up-to-date the information on the current projects in the area of food science in the country. A hand book of world wide institutional sources of food information has also been compiled to serve as a referral tool.

Services: Three categories of services are planned under NICFOS: (*i*) Information Service; (*ii*) Reprographic Service; and (*iii*) Translation Service.

Information Service

When fully established, NICFOS will be offering the following services:

1. Current awareness services (including new additions to the library)
2. Selective dissemination of information (SDI)
3. Abstracting of scientific and technical papers
4. Patent abstracts
5. Technical digest (in Indian languages also)
6. Management digest
7. Newsbrief (in Indian languages also)
8. Product information bulletin (in Indian Languages also)

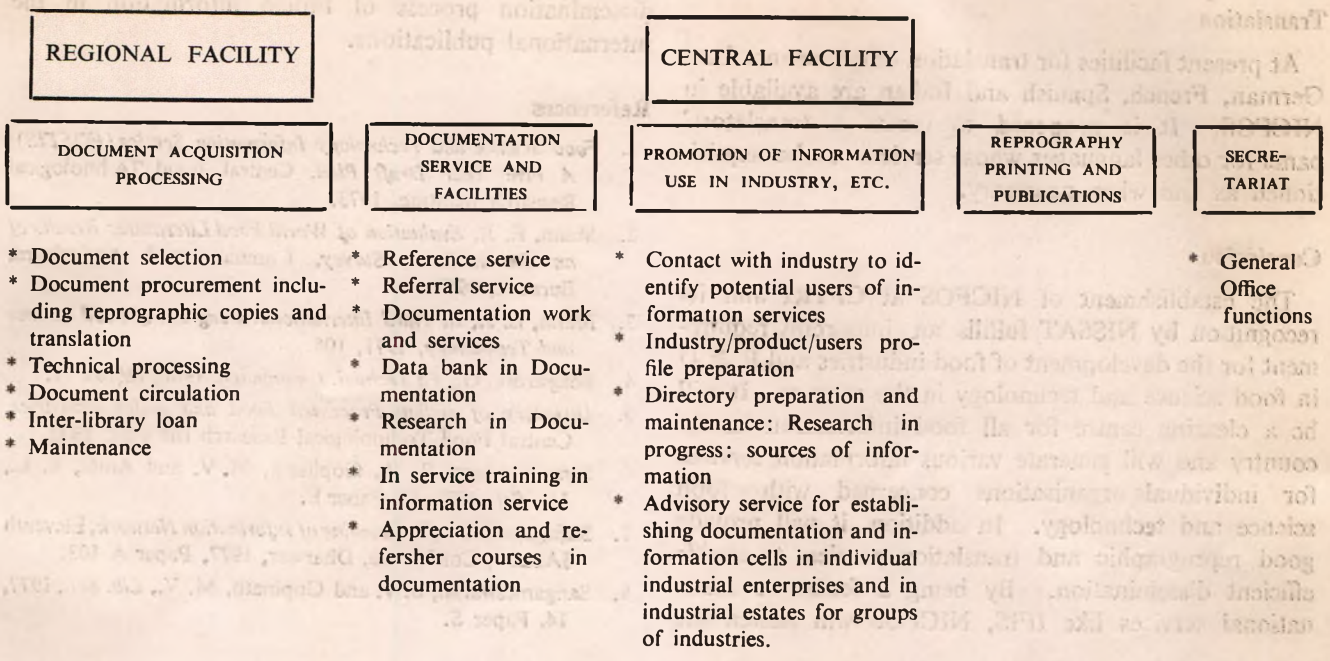

Fig. 2. NICFOS: Organisation Chart

9. Data compilation and service (scientific, technological and economic)

The current services of NICFOS include

Food Technology Abstracts: This is a monthly publication covering over 400 periodicals in food science and technology. Each issue includes about 300 abstracts/titles arranged in 18 sections along with a subject index. Each issue of Food Technology Abstracts also contains an accession list covering the new additions to the NICFOS library. This publication is intended to serve the specific information needs of the country.

Food Digest: This is a quarterly publication primarily oriented to the needs of managereal and technical personnel particularly in the food industries. Information in digest form on raw material, products, processes, equipment and food machinery, quality and legal aspects, techno-economic data etc. collected from diverse sources like periodicals, news letters, house journals, bulletins, trade journals, annual reports and news papers are included in this publication.

Food Patents: A quarterly publication covering information on food patents issued all over the world. Each issue covers about 100 patents, arranged section wise and is provided with a subject index.

SDI (manually operated): This is a personalised information service based on user profiles. At present, the service is given only to a few senior scientists of CFTRI. The periodicals soon after their receipt are scanned and the articles matching the profiles are sent to concerned users. Feed back from users facilitates modification of profiles.

Literature enquiry: NICFOS also handles enquires involving search of literature. A card index of literature included in other services of NICFOS has been maintained for this purpose.

Bibliographic service: Bibliographes are compiled on specific subjects from time to time particularly when conferences/seminars/symposia are held at CFTRI. So far, 22 bibliographies have been compiled. Bibliographies are also compiled on request.

Indian input to IFIS: NICFOS also collaborates with IFIS of UK as an input centre for Indian food information for inclusion in Food Science and Technology Abstracts.

Computerised information service: NICFOS proposes to use computers in generating SDI. For this purpose it is proposed to use international data bases. Preliminary work in this direction has started with the Computer Centre of IIT, Madras with encouraging results. A nation wide survey conducted has revealed that a considerable number of users are interested in this service. The soft ware requirements for this service and a thesaurus of food terms are being worked out at present.

Reprographic and Printing

These services are essential requirements for the supply of literature available in NICFOS to users for the proper dissemination of information. A well equipped reprographic unit with sophisticated xerox equipment and electronic scanners has been established. An offset printing machine supplemented by a process camera and plate making equipment is also available.

Translation

At present facilities for translation of documents from German, French, Spanish and Italian are available in NICFOS. It is proposed to create a translators' panel for other languages whose services can be requisitioned as and when necessary.

Conclusion

The establishment of NICFOS at CFTRI and its recognition by NISSAT fulfills an important requirement for the development of food industries and R & D in food science and technology in the country. It will be a clearing centre for all food information in the country and will generate various information services for individuals/organisations concerned with food science and technology. In addition, it will provide good reprographic and translation services to enable efficient dissemination. By being a feeder to international services like IFIS, NICFOS will hasten the dissemination process of Indian information in the international publications.

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BOOK REVIEWS

Advances in Biochemical Engineering, Vol. 10, *Immobilized Enzymes* 1 Edited by T. K. Ghose, A. Fiechter and N. Blakebrough, Published by Springer-Verlag, Berlin, Heidelberg & New York, 1978; pp. 177;

The book comprises five chapters on different aspects of the scope and application of the immobilized enzymes. This is a summary of the present knowledge of certain areas of enzyme engineering. The first chapter deals with recent advances in the design and operation of the immobilized enzyme reactors. Mass transfer and related effects, enzyme acrivity loss, cost estimates, reactor types and performance, operating strategy, economic considerations are discussed. General applicability of newer concepts are considered although specific practical examples are cited. As the author states, "reactor design and operation (for immobilized enzymes) is not a rapidly changing field and that most of the basic concepts have been established and remarkable gains in operating efficiency are not likely in most cases". The second one concerns with the kinetic analysis of immobilized multienzyme reaction systems and with specific sequential enzyme systems such as glucose oxidase and catalase starch degrading enzymes, α-and β amylase combination etc. and with immobilization of whole cells. The statement (p. 36, 11 23-24) that "One of the major industrial applications of immobilized enzyme technology is in the hydrolysis of starch" needs checking; and p. 88 1,1-4, p. 123 11.7 from bottom give a contrary picture! The author's speculation that "it is possible, however, from an industrial stand point the major area of interest will be the exploitation of immobilized whole cells" may become true in the case of most intracellular enzymes. A detailed treatment about the carriers is found in the third part. Carriers for immobilization of enzymes represent and extremely important and crucial factor in determing not only the efficacy and stability of the enzymes but also about the economic aspects. Various factors in the choice of a carrier, the environment of the carrier such as pH, surface propertiss, temperature, pressure, diffusion of substrates and cofactors, the carrier properties and structure are discussed satisfactorily. Treatment of carriers before immobilizing enzymes on them, immobilization methods by adaption and modifying surfaces of carriers are briefly and successfully described. The fourth chapter considers the potential and actual use of immobilized enzyme technology. It reviews the engineering aspects, preparation of immobilized enzymes (including whole cell systems), reactor design and performance, applications in food and pharmaceutical industry and in waste treatment. The last chapter purports to deal with starch hydrolysis by immobilized enzymes and industrial applications. About half the space is devoted to the well-known avoidable preliminaries on the chemistry of starch and enzyme hydrolysing starch. The rest deals with immobilized enzymes such as α and β-amylases and amyloglucosidase. Immobilized preparations of amyloglucosidase have been covered in some detail.

In general the book represents reviews on some important aspects of enzyme engineering and is useful. There are a few minor repititions which could have been avoided; e.g., reactors (1st chapter, 3rd chapter p. 84; 5th chapter, p. 151), preparation of immobilized enzymes (chapter 3, p. 78-80; chapter 5, p. 150-151). It would be indeed be helpful to the interested reader and researchers to know which industries actually use the immobilized enzyme technology and which ones are the most likely to employ this during the next 10 years or so ("prophesying" is hazardous but futurology is a respectable and necessary pastime). Perhaps touching the socio-economic aspects which may determine the applicability of these processes could also be desirable. For example, the desirability or otherwise of the process for high fructose syrup in the "sugar-rich" countries (India, France) vis-a-vis "sugar-poor" (USA and Japan). Inclusion of authoritative updated and comprehensive reviews on different aspects of enzyme engineering (even if it takes a whole issue or book) such as design and operation of reactors, carriers, immobilization, techniques, actual commercial processes and potential ones would be beneficial that is, there should be horizontal and vertical coverage of different aspects of enzyme engineering. Presently these are scattered in books, research journals and reviews many of which are excellent. Although "Immobilized enzymes are not the panacea for all enzyme applications that was initially projected in the late 1960s and the early 1970s" (p. 71, lines 1-16 from the bottom) a detailed status-of-the-art report is necessary. This present book fulfils part of these requirements.

M. R. Ragavendra Rao
C.F.T.R.I., Mysore.

Saving of Energy in the Production of Cold: International Institute of Refrigeration 177, Boulevard Malesherbes 75017, Paris, France; Pages-267 1979;

This book is a collection of 23 papers presented at the meeting of Commission B 2 of the I.I.R., held at Delft (the Netherlands), September 12-15, 1978. This Institute publishes the work of its Commissions as a series on "Refrigeration Science and Technology" and this volume is 43rd in the series. The volume contains 20 papers in English and 3 in French. Each carries a summary in the other language. The discussion following the paper, forms an important part of the technical matter in this volume.

The volume is very well organized. The first paper is an introductary lecture (in French) which sets the "boundary limits", so to say, for any energy saving exercises. Then follow 21 papers distributed in three sections, and lastly the concluding paper. The 21 papers are under 3 headings as follows:

1. Saving of Energy via improvements to current refrigeration systems (12 papers);
2. Integration of refrigeration in energy saving system (3 papers);
3. Less current systems and alternative sources of energy and cold (6 papers).

Each section has a sort of key-note paper which gives a broad overview and is then followed by others which deal with a specific area in detail.

The subject-matter is very well organized and present-papers seemed to be reporduced directly from the typed scripts as is evident from the variation in get up from paper to paper. However, it does not detract attention to the technical content.

It is an indication of the pragmatic approach that more than half of all the papers viz: 12 fall into the first section on saving energy via improvements to current refrigeration systems. The first keynote paper further classifies the energy saving ideas into those aiming at better operation procedures and housekeeping and requiring no capital expenditure. The other category carried out the saving through an energy audit and plant performance checks leading to plant modifications needing a moderate capital expenditure. The third category is based on extensive long term improvements in plant design.

Before the energy crisis, the objectives were standardization of components to reduce manufacturing costs and maintenance costs. There is, therefore, considerable scope for saving of energy by optimizing evaporator and condenser temperatures and other parameters. The other areas indicated are expansion valve systems *Vs* flooded evaporators, proper air circulation systems in cold storages, uniformity of load brought about by refrigeration storage (ice-bank concept). About half the papers in this section deal with compressor improvements. This section is very relevant to Indian conditions and is recommended to users as well as suppliers of refrigeration equipment.

The second section with three papers deals with integration of refrigeration systems with other energy systems to produce overall energy saving. The main themes are based on consideration of refrigeration as a heat pump system. If hot water is required, the heat thrown out of the condenser in the refrigeration system is utilized for heating or preheating water. The discussion brings out an important warning that what is optimized energy saving within the scope of the refrigeration industry may be wasteful in a larger context.

The third section of 6 papers dealing with other refrigeration systems not in current use and other sources of energy has a very interesting introductory paper. The other paper found interesting deals with non-azeotropic mixtures of refrigerants. Mixing of two refrigerants of different boiling points gives the possibility of changing the capacity of the refrigeration system continuously or as desired by changing the proportion of the two refrigerants. An overall improvement in coefficient of Performance is expected this way. The papers deal with use of solar and other forms of energy. They would not be of immediate interest to Indian conditions even though they refer to some as suited to the developing countries.

The concluding paper puts the energy crisis in a proper time perspective in relation to the age of the planet and the relatively short time span of the current technological civilization and follows with high-lights of most of the papers.

Apart from being an interesting and useful set of papers on energy saving, I strongly recommend this book to all our numerous professional bodies as an example of how a professional body can organize study commissions and publish the technical matter in a useful form.

S. S. KALBAG
HINDUSTAN LEVER LTD., BOMBAY.

Coffee Pulp—Composition, Technology and Utilization: by J. E. Braham and R. Bressani (Ed.), International Development Research Centre, Ottawa, Canada K1G 3H9, 1979 pp. 95, Price: $ 54.

This monograph covers the following aspects of utilisation of the coffee pulp. In 10 Chapters, subjects like chemical composition, potential uses of by-products, use of coffee pulp in ruminant feeding, swine feeding, coffee-pulp as silage, processing and chemical treatments and antiphysiological factors are covered by various authors. These areas are well documented. Attention is focussed on utilization of the coffee pulp which forms about 25-30 per cent of the coffee berry.

The pulp is high in fibre content of about 20 percent, crude protein 10 per cent and ash constituent of 8.5 per cent, High potassium content of 1765 mg per cent can impair the use of coffee pulp as animal feed. The various uses of coffee pulp can be as animal feed, as ensilage and as a partial source of caffenie, protein and organic fertilizer. It can also be used as a source of pectic enzyme, concentrated coffee pulp extract and vinegar. Various types of microorganisms have been grown in the material. Biogas has also been produced along with cow manure.

Coffee pulp first came into attention as animal feed, but discouraging results were obtained in its use as a cattle feed. The total and digestible nutrient content of coffee pulp shows that this agricultural by product can impart nutrition similar to that of good quality forages. The digestibility of ensiled pulp is good but due to the high level of lignified nitrogen the availability of protein is affected. Upto 20 per cent, the response is favourable. At concentration higher than 20 per cent, lowering of nitrogen utilization was observed. The palatability is not so good. The ensiled coffee pulp was better than dehydrated pulp due to its better palatibility, better digestibility and perhaps lower content of caffeine and tannin. There is still a need to determine long term effects of coffee pulp on the productivity of beef and dairy herds. For swine feeding upto 16 per cent diet no detrimental effect was noticed. Since chicken do not tolerate high amount of fibre in the feed coffee pulp as a constituent of poultry ration does not appear to be promising. Coffee pulp depressed growth, decreases feed efficiency in chicken and resulted in mortality also. Studies have shown ensiling can be used for conversion of coffee pulp as cattle feed. Pretreatment will be essential to remove undesirable constituents. Coffee pulp silage is a better method of conservation and preservation.

Fresh pulp is an abundant by product in coffee industry, but as a value in animal feed is limited by the presence of anti-physiological factors such as caffeine, tannins, chlorogenic acid, caffeic acid and excess of potassium. Various treatments like calcium hydroxide, sodium metabisulphite, water and sodium hydroxide have been tried to remove toxic compounds. Considerable studies are still needed to identify all toxic factors. Pretreatment will help in upgrading the material and ensilaging will result in better utilisation on cattle feed to a varying extent.

Coffee pulp has potential nutritive value as a cattle feed but the digestibility is not very good. Ensilaged pulp appears to be superior to that of dehydrated pulp as a nutrient. Availability of protein, however, in both cases is affected by high levels of lignified nitrogen. Ensilage reduces levels of toxic factors as caffeine increases digestibility. Suitable microorganism to detoxify the toxic factors should be isolated to upgrade this material.

The monograph has been compiled with considerable data along with bibliography.

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

Nutritious Food Supplements for Infant and Preschool Children: Published by the Association of Food Scientists and Technologists (I), Madras Chapter, No. 8 Gopalapuram, Ist street, Madras-600 086, 1979, pp. 73, Price: Rs. 5.00.

This publication brought out on the occasion of the Symposium on Food Needs of Infants and Preschool Children held in Madras contains recipes for indigenous infant foods and food mixes. Each receipe and food mix contains information on ingredients, processing and nutritional value of the product. Seventeen food mixes and food recipes developed at Coimbatore, Gandhigram, Hyderabad, Poona and West Bengal have been covered.

ASSOCIATION NEWS

Our New Editor

The Executive Committee of the Association of Food Scientists and Technologists (India), is happy to announce the nomination of Dr. R. Radhakrishnamurthy as the New Editor of the Journal of Food Science and Technology. He is taking charge from 1st January 1980 for a period of 3 years.

Dr. R. Radhakrishnamurthy had his initial academic training at Andhra University, Waltair where he graduated with B.Sc. (Hons) in Chemistry. During 1949–50 he was a research student in the Fermentation Technology Division of Indian Institute of Science, Bangalore. From 1951–'57, he was a research assistant at the University of Biochemical Laboratory, University of Madras and obtained Ph.D. degree in Biochemistry from University of Madras in 1956. In 1957, he joined the Biochemistry department of Central Food Technological Research Institute, Mysore and is continuing his research activities over the last 22 years. During 1965–'67 he worked as a post doctoral research fellow of the National Research Council of Canada in the Department of Nutrition, University of Toronto, Toronto, Canada. He is interested in the areas of Nutritional biochemistry, and pesticide biochemistry.

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Research Papers

Research Notes

*Vol. 17, Nos. 1 & 2 will be Prof. V. Subrahmanyan Commemoration issue containing invited articles.

Printed and published by J. D. Patel, Secretary, AFST (India), CFTRI, Mysore-570013, at Sharada Press, Mangalore-575001

INSTRUCTIONS TO CONTRIBUTORS

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.

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

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

   In the text, the references should be included at the end of the article in serial order.

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

   (*a*) *Research Paper:* Menon, G. and Das, R. P., J. sci. industr. Res., 1958, **18,** 561.

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

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

   (*d*) *Proceedings, Conferences and Symposia:* 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.

ANNOUNCEMENT

Dear Member,

The new Bye-laws of AFST(I) come into effect from Ist January 1980. As per the Bye-laws, the membership fee should be paid on or before 31st March of each calendar year. All the members are requested to pay their membership fee as early as possible so that they may not miss any issue of our Journal.

The revised membership fee is as follows:

Mode of membership	Fee	Admission Fee
Life Member	Rs. 300-00	Rs. 2-00
Corporate Member	Rs. 300-00	Rs. 5-00
Members (Ordinary)	Rs. 20-00	Rs. 2-00
Affiliate Member	Rs. 30-00	Rs. 2-00
Student Member	Rs. 10-00	Re. 1-00

There is no change in the existing subscription rates.

It is also requested that the arrears, if any, may also be cleared.

HON. EXC. SECRETARY.