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CONTENTS

Research Papers

Research Notes

RESEARCH PAPERS

Studies on the Microbiological Quality of Traditional Indian Sweetmeat Products

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Microbiological quality of fresh, stored and sugar syrup soaked sweets procured from the local market was studied. Aerobic mesophiles and thermophiles, mesophilic and thermophilic acid producers and mesophilic and thermophilic spores were observed in varying numbers in all the types of samples studied. No definite correlation could be established between the different categories of the sweet preparations excepting sugar syrup soaked types which recorded higher counts of aerobic mesophiles and thermophiles.

Fresh *Dudh Pedha*, stored *Dudh Burfi* and all the samples of sweets soaked in sugar syrup excepting *Jelabi* and *Jangree* were found to be contaminated with coliforms whereas, only stored samples of *Dudh Pedha* and *Dudh Burfi* indicated the presence of coagulase positive *Staphylococci* and *Salmonella* respectively.

Many kinds of traditional sweetmeat products are being manufactured and sold under varying conditions in our country. No authentic data are available to indicate exactly the volume of the trade, because the industries manufacturing these types of preparations are not well organized. Unofficial reports indicate that in Bombay city alone there are over 700 dealers having an annual turnover of well over ten million rupees. Date *et al.*[1] have reviewed the problems involved in the preservation of Indian sweets. Gatlewar *et al.*[2] have carried out bacteriological analysis of sweetmeats sold in Bombay and have found that nearly 47 per cent of the samples studied contained enteropathogenic bacteria and only 35 per cent were found to be free from bacteria. Mokashi *et al.*[3] in their studies on bacteriological analysis of ice cream sold in Bombay, have found that only 5 per cent of the samples were free from bacteria. The rest of the samples showed coliforms of faecal origin and, further, most of the *Escherichia coli* strains isolated were found to cause food poisoning and diarrhoea. Kamat and Sulebele[4] have carried out microbiological analysis of *Pedha* samples collected in Bombay city and have reported staphylococcal contamination. Ajab Singh *et al.*[5] have surveyed the microbiological quality of *Burfi* and *Pedha* samples in the Allahabad market, and reported extensive coliform and staphylococcal contamination of the product. Coliform and staphylococcal contamination have been found to be more in *Pedha* than in *Burfi* samples. Kirshan Singh *et al.*[6] have examined the microbial quality of indigenous concentrated milk products such as *Khoa*, *Khurchen*, *Pedha* and *Rabbri* from Agra market and have reported that *Rabbri* had the highest microbial count.

In this paper, data are presented relating to the microbiological quality of market samples of traditional Indian sweetmeat products (fresh, stored, and types of sweets soaked in sugar syrup).

Depending on the raw materials used in the preparation of sweets, the samples used in the present study could be broadly grouped into:

Bengal gram (*Cicer arietinum*) flour based:

Mysore Pak: A porous structured sweet prepared by heat processing a mixture of Bengal gram flour, hydrogenated fat and sugar.

Sohan Pappadi: Laminated structured sweet prepared by heat processing a mixture of Bengal gram flour, refined wheat flour and hydrogenated fat. Fibrous structure is formed due to crystallization of sugar during processing. The product is generally topped with broken cashew kernels and raisins.

Sweet Boondhi: Fat fried product having beaded appearance. The final product is allowed to soak in sugar syrup.

Refined wheat flour (*Maida*) based:

Badam Puri: A product made out of refined wheat flour and hydrogenated fat. The final product is allowed to soak in sugar syrup.

Jelabi: Fat fried product prepared out of refined wheat flour with a small proportion of Bengal gram

flour. The final product is allowed to soak in sugar syrup.

Milk based:

Dudh Pedha: Dry sweet prepared from *Khoa* (desiccated milk) and sugar.

Dudh Burfi: Prepared by processing a mixture of *Khoa* and sugar syrup.

Champakali: Prepared out of milk after curdling with tartaric acid. The milk solids are made into desired shapes and cooked in sugar syrup. The product is stuffed with sugared *Khoa* and topped with cherry fruit.

Kalakand: prepared out of milk after precipitating with tartaric acid. Milk solids so obtained are then cooked in sugar syrup. It has a pasty appearance.

Apple Sweet: Prepared out of milk and is very much similar to *Champakali*, but it is shaped and coloured differently with the only difference that the apple sweet is not stuffed with sugared *Khoa*.

Miscellaneous types

Jangree: Prepared by grinding together black gram dhal (*Phaseolus mungo*), rice and Bengal gram dhal. The batter is allowed to ferment overnight and fried in fat and finally soaked in sugar syrup.

Materials and Methods

Collection of samples: Duplicate samples of the following kinds of traditional Indian sweetmeats were collected at random from the local market in sterile containers and the samples were processed within one hour of its collection. The different samples collected were as follows:

Fresh and stored samples of *Mysore Pak*, *Sohan Pappadi*, *Badampuri*, *Dudh Pedha* and *Dudh Burfi*.

Sugar syrup soaked fresh samples of *Champakali*, *Jelabi*, *Kalakand*, *Apple sweet*, *Jangree*, and *Sweet Boondhi*.

Preparation of the sample: Fifty grams of the sample was blended for 2 min in a sterile blending container with 450 ml of sterile buffered phosphate diluent to obtain 1/10 dilution. Decimal dilutions were prepared upto 1/10000. The samples were processed for aerobic mesophiles, thermophiles, mesophilic and thermophilic spores, mesophilic and thermophilic acid producers, yeasts and moulds, MPN-coliforms, MPN-*Escherichia coli*, MPN-Enterobacteriaceae, coagulase positive *Staphylococci*, *Salmonella* and haemolytic *Streptococci*, as per methods described by APHA[7], Thatcher and Clark[8] and Heidelbaugh, *et al.*[9]

Results and Discussion

Fresh sweetmeat samples were procured within 24 hours of their preparation. The data relating to these types are presented in Table 1. As seen from the Table 1, aerobic mesophiles, thermophiles, mesophilic and thermophilic spores, mesophilic and thermophilic acid producers, yeasts and moulds were present in varying numbers. The maximum count for aerobic mesophiles was of the order of 5000/g in *Dudh Pedha* and the least count was observed in *Mysore Pak*. *Mysore Pak* is a sweet which is rich in fat and sugar and low in moisture content (2.7 per cent) so that the product cannot support high microbial growth. The aerobic mesophiles in all the samples were found to be more than the mesophilic spores with the exception of *Mysore Pak*. Such a high survival of mesophiles in an environment rich in fat and sugar may be partly due to their adaptability or dilution of sugar on the surface by condensed moisture. Aerobic thermophiles were completely absent in *Badam Puri*. Aerobic mesophilic and thermophilic

TABLE 1. ANALYSIS OF FRESH SWEETMEAT SAMPLES

Organisms/Composition/g	Mysore Pak	Sohan Pappadi	Badam Puri	Dudh Pedha	Dudh Burfi
Mesophiles	200	1.0×10^3	2.6×10^3	5.0×10^3	400
Thermophiles	2.4×10^3	110	NIL	2.5×10^3	420
Mesophilic acid producers	200	900	20	400	NIL
Thermophilic acid producers	450	40	NIL	50	,,
Mesophilic spores	NIL	NIL	,,	10	100
Thermophilic spores	,,	,,	,,	148	120
Yeasts and moulds	510	820	12.6×10^3	14.2×10^3	10.0×10^3
Coliforms	NIL	NIL	NIL	460	NIL
Moisture, %	2.7	3.2	9.6	11.3	12.0
Sucrose, %	50.3	38.3	35.6	59.4	40.8

E. coli, Enterobacteriaceae, coagulase positive *Staphylococci* and haemolytic *Streptococci*, were not observed in any of the above samples.

TABLE 2. ANALYSIS OF STORED SWEETMEAT SAMPLES

Organisms/Composition/g	Mysore Pak	Sohan Pappadi	Badam Puri	Dudh Pedha	Dudh Burfi
Mesophiles	340	750	2.5×10^3	760	520
Thermophiles	1.0×10^3	65	100	680	600
Mesophilic acid producers	Nil	90	200	600	Nil
Thermophilic acid producers	,,	35	15	80	,,
Mesophilic spores	250	Nil	1500	40	300
Thermophilic spores	810	15	85	50	390
Yeasts and moulds	Nil	1.0×10^3	65×10^3	94×10^3	16.1×10^3
Coliforms	,,	Nil	Nil	Nil	161×10^3
Staphylococci	Nil	Nil	Nil	240	Nil
Moisture, %	1.4	3.1	8.8	7.4	10.0
Sucrose, %	55.6	38.8	35.6	45.7	40.8

E. coli, Enterobacteriaceae, *Streptococci* were absent in all the above samples, *Salmonella* was absent in all samples except in *Dudh Burfi*, where it was positive.

spores were 10 and 148/g in *Dudh Pedha* and 100 and 120/g in *Dudh Burfi* respectively. Aerobic mesophilic and thermophilic acid producers were present in the range of 20-900/g and 40-450/g respectively. The aerobic mesophilic count has limited validity as to microbiological safety of the product. However, a high aerobic mesophilic count will often indicate contaminated raw materials, unsatisfactory sanitation or unsuitable time/temperature conditions during processing, transport and storage of the product. Similarly the presence of aerobic thermophiles would indicate thermophilic contamination from equipment contact surfaces such as blanching equipment or from heated pumps in food conduit system. Since no such equipments are used in the processing of sweetmeat products, the only possible source for thermophiles including the acid producers would be the sugar that is used. There are no microbiological standards available for these types of products, excepting for *Burfi* (preparaed out of *Mawa* as well as other types). The Indian Standards Institution has stipulated standards in respect of standard plate count of 30,000/g and yeast and mould count of 10/g.

The most significant aspect of microflora of sweetmeats is the high incidence of yeast and mould counts which ranged between 510 and 14,200/g for the various sweets. Yeasts and moulds could not have survived the heat processing received during manufacture of these products. The only possible source would be secondary sources of contamination when the products are allowed to solidify, in most cases uncovered. It is relieving to note the complete absence of organisms of public health

TABLE 3. ANALYSIS OF SWEET SAMPLES SOAKED IN SUGAR SYRUP

Organisms/Composition/g	Champakali	Jelabi	Kalakand	Apple Sweet	Jangree	Sweet Boondhi
Mesophiles	97×10^4	760	32.6×10^4	14.1×10^4	200	1.17×10^4
Thermophiles	6.9×10^4	4.0×10^4	33.6×10^4	10.2×10^4	100	1.2×10^3
Mesophilic acid producers	252	700	182	841	Nil	6.0×10^3
Thermophilic acid producers	68	24	700	68	60	30
Mesophilic spores	35	145	10	172	80	20
Thermophilic spores	141	78	3.2×10^3	131	Nil	110
Yeasts and moulds	580	Nil	38.8×10^3	Nil	30.0×10^3	55.6×10^3
Coliforms	23	,,	4	1.0×10^3	Nil	9
Escherichia coli	Nil	,,	Nil	Nil	,,	2
Moisture, %	32.2	17.2	17.4	30.7	13.7	18.9
Sucrose, %	53.2	45.7	35.7	51.8	49.6	46.9

Enterobacteriaceae, *Staphylococci*, *Streptococci* and *Salmonella* were not detected in any of the above samples.

significance such as members of the group of Enterobacteriaceae, *Salmonella*, coagulase positive *Staphylococci* and haemolytic *Streptococci*. However, two samples of *Dudh Pedha* indicated the presence of coliforms.

The stored sweetmeats were procured from the shops which were in isolated localities where the sweetmeats had undergone varying periods of storage under the conditions existing in the shops. The microbial counts relating to these types of samples are presented in Table 2. The counts for stored sweetmeats could not be compared with those for fresh ones due to wide variations between the two types. It is significant to note that the yeast and mould count had increased manifold in most of the sweetmeats examined. This may be probably due to direct exposure of these products to atmosphere during storage.

Only one sample of *Dudh Burfi* indicated suspected Salmonella contamination which, incidentally, had very high count of coliforms. Among the many stored sweets examined, *Dudh Pedha* indicated coagulase-positive staphylococcal contamination. Similar observations have also been made by Kamat and Sulebele.[4] The reason for such a type of selective contamination is not clear at the moment.

Some of the sweets such as *Champakali*, *Jelabi*, *Kalakand*, *Apple Sweet*, *Jangree* and *Sweet Boondhi* are more juicy in nature because these sweets are soaked or cooked in sugar syrup compared to other types of sweets which are of relatively dry nature. It was of interest to study whether the incidence of microflora in respect of these types of sweets is different from other types of sweets. The data obtained are presented in Table 3. As seen from the Table, aerobic plate counts of all the sweetmeat samples were quite high excepting that of *Jangree*. Aerobic plate counts in general were higher in these types because of high moisture content compared to the other types of sweets which are drier. Similarly, coliforms and yeasts and moulds were present in all the sweets excepting *Jelabi* and *Jangree*. Mesophilic and thermophilic spore formers were lesser in number compared to total aerobic mesophiles and thermophiles. Organisms of public health significance were totally absent in these types of sweets.

As was expected, it was observed that those sweet preparations that are soaked in sugar syrup and have higher moisture contents supported better microbial growth compared to samples having lesser moisture content.

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Effect of Milk Coagulants on the Quality of *Chhana* and *Chhana* Whey

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The effects of three milk coagulants, namely, citric acid, lactic acid and sour *chhana* whey on the quality of *chhana* were studied. While citric acid did not impart any flavour to *chhana* and produced soft and smooth body and texture, lactic acid and sour *chhana* whey resulted in slightly sour taste, acidic flavour and hard, granular body and texture. Highest and lowest recoveries of *chhana* were obtained by using sour whey and citric acid as milk coagulants respectively. Total solids, fat, protein, soluble extract, water soluble free fatty acids and acidity in *chhana* were found to vary from coagulant to coagulant. Milk coagulants did not affect the *chhana* whey composition significantly except in respect of acidity.

Chhana is a solid milk product obtained by acid coagulation of milk followed by removal of whey[1]. Fairly large amount of milk produced in West Bengal, Orissa and Bihar is converted into *chhana*. While whey is mostly thrown away, *chhana* is used exclusively as base for the preparation of choicest sweetmeat delicacies like *Rasogolla* and *Sandesh*. The nature and type of coagulant, temperature, pH of coagulation and the quality and composition of milk are some of the important factors which control the physical and chemical nature of *chhana*[1-3]. This paper presents the results of an investigation of the effect of milk coagulants on the yield and physico-chemical properties of *chhana* and its whey.

Materials and Methods

Cow milk from the Institute herd was used for *chhana* preparation by using citric acid, lactic acid and sour *chhana* whey (aged *chhana* whey) as milk coagulants. The two organic acids were used at a concentration of 1.5 per cent for *chhana* preparation in the experiment. The average acidity of sour whey used for *chhana* preparation was about 1.6 per cent in terms of lactic acid. About 2.5 kg milk divided into three groups of equal volume was used in each experiment. Milk was heated with frequent stirring. When milk started boiling, it was removed from heater and coagulant added slowly from a burette and stirred continuously till the milk coagulated completely. Whey was removed by filtering on muslin cloth for about 2.5 hr. The physical characteristics of *chhana* samples were examined in respect of colour, flavour, body texture and taste.

Total solids and total soluble extracts were determined according to Davis and Macdonald[4] and the acidity was estimated by the method described in AOAC for cheese.[5] Protein and fat contents of *chhana* were determined according to the ISI method described for canned *Rasogolla*.[6] Water soluble free fatty acids (WSFA) of the *chhana* was determined by treating the water extract with petroleum ether (40-60°). The ether extract was dried and weighed.

Lactose and protein contents of *chhana* whey were determined by iodometric and Kjeldahl methods respectively.[4] Total solids and acidity were determined by standard procedures.[5] Fat content was estimated by the ISI method.[7] Six trials on the effect on milk coagulants on the quality of *chhana* and its whey were carried out.

Results and Discussion

Effect of milk coagulants on the physical properties of chhana: Treatment of milk with citric acid produced the *chhana* of soft and smooth body and texture devoid of any odour. On the other hand, lactic acid and sour whey produced slightly acidic and sour *chhana* with hard and granular body and texture. Light creamy colour of the *chhana* was obtained by using all the three milk coagulants.

Optimum conditions of coagulation: Higher pH (5.77) and lower temperature (81.6°C) of coagulation were observed for citric acid as compared to other milk coagulants. The pH (5.52) and coagulation temperature (83.6°C) were comparatively lower and higher respectively in the case of sour whey. The corresponding values for lactic acid were found ot be 5.70 and 82.8°C. It was also observed that the coagulation temperature was related to the amount of coagulant required. Higher amount of citric acid as compared to that of lactic acid was required for complete coagulation (Table 1). The required amount of sour whey depended upon its acid content.

TABLE 1. AMOUNT OF MILK COAGULANT REQUIRED FOR CHHANA PREPARATION

Coagulant	Acidity (as % lactic acid)	Acid strength (N)	Coagulant/100 ml milk (ml.) Mean±S. D.
Citric acid	0.99	0.198	71.8±4.18
Lactic acid	1.10	0.220	64.6±5.01
Sour whey	1.6	—	58.9±8.48

Recovery of chhana: On economic grounds, recovery of *chhana* has always been a matter of great interest. We observed 20 per cent recovery in the case of sour whey as milk coagulant while about 19 per cent yield was obtained in both the cases of lactic and citric acids. Similar studies by De and Ray[1] and Srinivasan and Anantakrishnan[3] reported lower yield of *chhana* using citric acid as coagulant. However, slightly higher yield was also reported using the same coagulant.[8] Mahanta[9] had reported a 20 per cent recovery of *chhana* from cow milk using citric acid.

Composition of chhana and its whey: Tables 2 and 3 show the chemical composition of *chhana* and its whey prepared by different milk coagulants. It is apparent from Table 4 that lactic acid and sour whey as milk coagulants produced highest and lowest total solids respectively. Dey and Ray[1] reported higher total solids in *chhana* using the same milk coagulants as compared to the yields of this study. Srinivasan and Anantakrishnan[3] have also reported higher total solids (46.6 per cent). However, Balasubramanian and Basu[10] have reported a value of 42.9 per cent for total solids which is very similar to the results obtained in the present investigations. These variations are within the range of 5-10 per cent and can be explained on the basis of quality of milk used for the *chhana* preparation by various workers.

TABLE 2. EFFECT OF MILK COAGULANTS ON THE CHEMICAL COMPOSITION OF CHHANA

Constituents	Citric acid Mean±S.D.	Lactic acid Mean±S.D.	Sour chhana whey Mean±S.D.
Total solids	41.70±0.87	42.41±0.37	41.23±0.82
Fat	23.15±0.72	23.21±0.94	22.86±1.06
Protein	17.41±0.11	17.43±0.11	17.29±0.03
Total soluble extract*	15.24±0.15	14.42±0.31	14.63±0.39
Water soluble FFA*	0.10±0.01	0.11±0.01	0.12±0.01
Acidity**	39.00±4.80	37.00±7.40	39.00±8.00

*Free fatty acid; **ml. 0.1N/NaOH/100g *Chhana*

Values are expressed as percent of strained wet *chhana*. Calculated on the basis of moisture free *chhana*.

TABLE 3. COMPOSITION OF WHEY OBTAINED DURING PREPARATION OF CHHANA

Whey constituents	Citric acid Mean±S.D.	Lactic acid Mean±S.D.	Sour chhana whey Mean±S.D.
Total solids %	6.58±0.28	6.65±0.23	6.69±0.26
Protein %	0.61±0.84	0.63±0.07	0.63±0.04
Fat %	0.31±0.08	0.33±0.07	0.36±0.07
Lactose %	5.18±0.22	5.05±0.16	5.15±0.17
Acidity %	0.23±0.09	0.23±0.06	0.19±0.04

The fat and protein contents of the *chhana* samples prepared by using all the three milk coagulants were observed to be about 23 and 17 per cent respectively. De[2] and Srinivasan and Anantakrishnan[3] reported slightly higher fat content in *chhana* prepared by citric acid. However, Balasubramanian and Basu[10] had reported lower values (20 per cent) in similar experiments using the same milk coagulant. Values obtained for protein content in the present investigation compare very well with those reported by other workers.[2,3]

Citric acid as milk coagulant resulted in higher soluble extract and lower water soluble free fatty acids as compared to other milk coagulants used. Lowest soluble extract was observed in *chhana* prepared with lactic acid and sour whey coagulated *chhana* was found to contain higher water soluble free fatty acids.

Acidity was found to be slightly lower in the *chhana* prepared with lactic acid as compared with other milk coagulants.

In the *chhana* whey prepared with sour whey as milk coagulant contents of total solids, protein and fat were higher and the acidity were lower as compared to those in the whey prepared with other milk coagulants (Table 3). Lactic acid produced lowest lactose content in the whey. In a similar experiment other workers[3,11] have reported a value of lactose content in *chhana* whey very close to that obtained in these investigations.

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Production of Processed Cheese Using a Flavour Concentrate Prepared by Cream Fermentation

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A rapid method for the manufacture of processed cheese is described, based on integrated steps comprising of production of flavour by cream fermentation using *Candida lipolytica* var. *planta*, preparation of bland curd from standardised or skimmed milk, and, melting the curd along with flavour, colour and salts to produce processed cheese. The data given are based on trials in a small plant designed to produce about 25 tonnes per month of processed cheese. In brief, the method consisted of growing the yeast in cheese whey medium to produce cell-bound lipase, incubating the cream with cells to produce flavour compounds, chiefly fatty acids, and, using this cream to produce the processed cheese from freshly prepared curd. The cheese-flavoured cream could be stored at 4-6°C upto a month without deterioration in flavour quality.

A process for the development of cheese-like flavour by fermentation of the cream with *Candida lipolytica* strain C_1B was described earlier.[1-3] The flavour was produced through the lipolytic action of a cell-bound lipase on fat in cream and was very reminiscent of cheddar cheese. The unique advantage of the approach was that the process itself was suitable for the Indian situation where production of the cheese was not a major industry and unnecessary investment on long term ripening times could be avoided, ensuring at the same time a closer control over the quality of the processed cheese. The total time required for the process was three days for the production of flavour and one day for the preparation of the processed cheese as compared with 6-8 months required for production of cheese by conventional method. In the present paper, a complete method is described for the manufacture of processed cheese based on experience gained during actual plant trials.

Materials and Methods

Strain and media: Candida lipolytica var *planta* strain C_1B, composition of tomato-tryptone agar (TTA) and cheese whey medium, and analytical methods used for assay of enzymes, viable count, free fatty acids (FFA), fat and proteins (acid soluble and insoluble) have been as described earlier.[1,2]

Flavour production: Strain C_1B was grown on TTA in Roux bottles at 25°C for 48 hr. Cells from each of the Roux bottles were suspended in 100 ml of Ringer's solution and pooled suspensions were inoculated in 60 l. of cheese whey medium in a 100 l. capacity fermentor to give an initial cell concentration of about 3×10^7 per ml. It was grown at 25°C for 30 hr with aeration (500 ml/min) and stirring (300 rpm). Final concentration of cells in growth fermentor ranged $50\text{-}65 \times 10^7$ per ml with a lipase activity of about 900-1100 units per mg dry weight of cells. The culture was then transferred to the cream fermentor (500 l. capacity) for production of flavour. The inoculum was optimised at about $2\text{-}3 \times 10^{11}$ cells per kg of fat in cream and incubated for 30 hr at 30°C with constant stirring, but no aeration. The end-point of fermentation was determined organoleptically and by estimation of FFA which reached about 28-30 milliequivalent per 100 g of fat.[2] All operations

were carried out under total aseptic conditions. The flavoured cream was stored at 4°C in polythene bags till use.

Preparation of cheese curd: Basic steps involved in the preparation of bland curd are well-known.[4] Skimmed or standardised milk was acidified to pH 6.0 (0.25-0.3 per cent acidity) and treated with Hansen's rennet (2.5 g per 100 kg of milk) for 20 min till the curd became firm. It was then cut into 1 cm cubes and the temperature was raised to 45°C with constant but gentle stirring. They were held at this temperature till they became firm. They were then piled at the bottom of the renneting vessel and whey collected for use in growth fermentor. The piled curd was pressed through the cheese cloth till the moisture was reduced to 40-45 per cent, shredded, milled and used for the preparation of processed cheese.

Results

A complete flow-sheet for the production of 25 tonnes of processed cheese per month is given in Fig. 1. Basically it consists of two operations: (*a*) fermentative production of cheese flavour, and (*b*) preparation of the bland curd from skimmed or standardised milk. Both these operations could be carried out independent of each other with the result that the total time required for the whole process was less than three days.

Fermentative production of cheese flavour is essentially a two-step process involving growth of the strain C_1B in cheese medium to produce lipase, and incubation of the cells and lipase with cream to produce cheese flavour. The fermentors were of conventional design[5] made of double-jacketed stainless steel vessels provided with an arrangement for sparging air, stirrer, sampling device and a thermostatic control. The major difference between the growth and the cream fermentors was that anchor type stirrer was used in latter case (Fig. 1). Furthermore, there was no necessity to aerate the cream during fermentation. For aeration in growth fermentor, it was extremely important to use air completely free from any odoriferous (eg. oil from compressor) vapours because of the very sensitive property of the cream to pick up even the slightest of odours. Because of this, a watering air pump was used as a source of air which was passed through a water separator, exchanger, chilling unit to remove residual moisture, and then finally sterilised by passing through air filters before introduc-

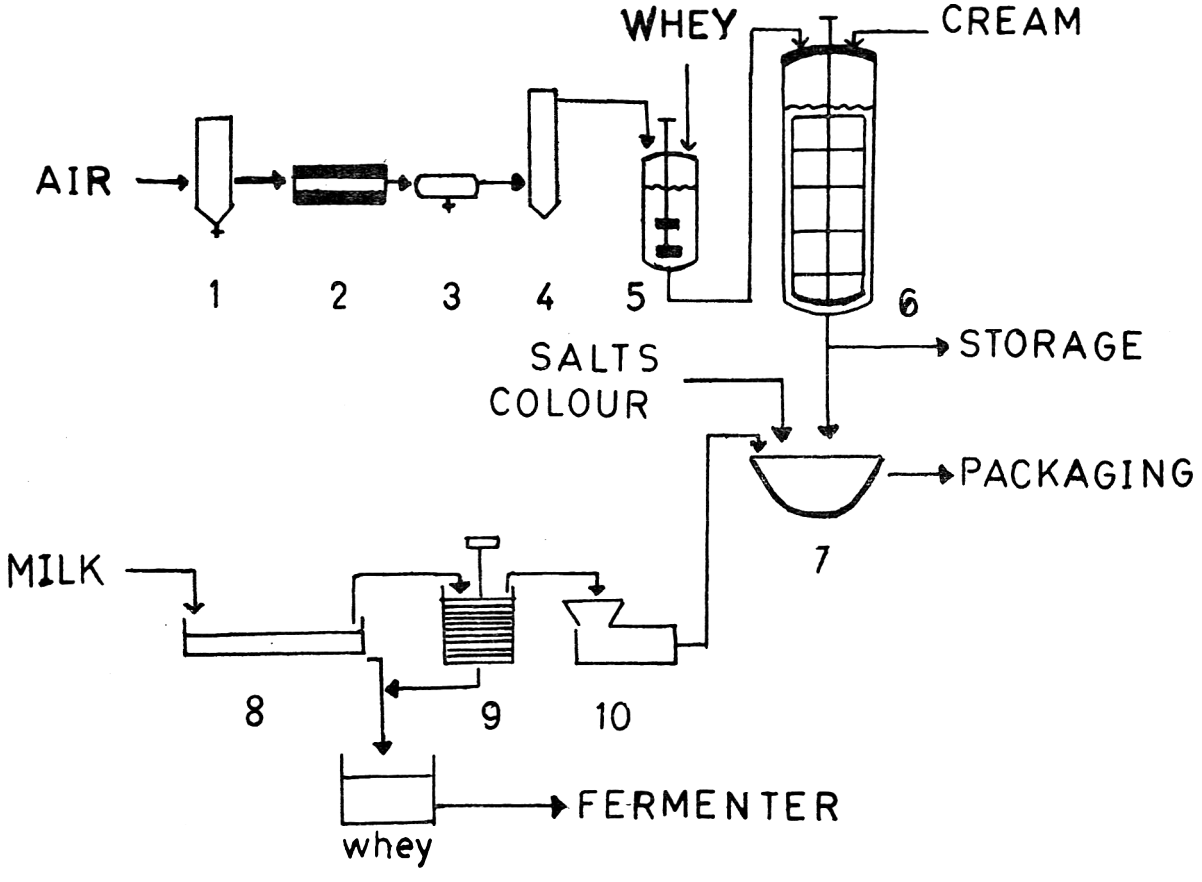

Fig. 1. Flow diagram for the production of processed cheese with flavour concentrate of cream fermentation.

(1) Cyclone water separator; (2) plate heat exchanger; (3) chilled water condensor; (4) filter for sterilisation of air; (5) growth fermentor; (6) cream fermentor; (7) Kustner melting kettle; (8) renneting vat; (9) cheese press; (10) shredder & milling machine.

TABLE 1. PLANT TRIALS ON PRODUCTION OF FLAVOURED CREAM

Batch No.	Growth of yeast				Fermentation in cream					
	Volume (litres)	Inoculum (cells/ml $\times 10^7$)	Final (cells/ml $\times 10^8$)	Lipase (units/ml)	Quantity (kg)	Fat (%)	Fat total (kg)	Cells/kg fat $\times 10^{11}$	Lipase (units/kg fat) $\times 10^4$	FFA(meq/ 100g fat)
1	70	4.4	6.5	58	200	73	146	3.1	2.76	36.05
2	70	4.9	5.85	72	200	76	152	2.7	3.3	32.00
3	50	4.5	6.2	68	105	86	90.5	2.67	3.75	30.58
4	63	5.6	5.0	98	160	67.5	108	3	3.85	29.20
5	50	2.9	5.35	104	200	65	138	2	2.6	40.00

ing into the fermentor. The aseptic conditions during fermentation and culture transfers were ensured by maintaining constant positive pressure with sterile air in both the fermentors.

Data on some of the routine plant trials are given in Table 1. Yields of cells as well as the lipase activity in fermentors were superior to those obtained in laboratory trials.[2] Absolute sterility in the growth fermentor was essential since many of the known aerobic contaminatns are strongly proteolytic and contribute products which produce off-flavours in cheese. Since lipase was cell-bound in this strain, the inoculum was standardised in initial studies in terms of number of cells in growth medium. However, subsequently it was observed that there were some variations in lipase yields in different batches, possibly because of its inducible nature.[1] It was, therefore, necessary to standardise the inoculum for the cream in terms of amount of lipase per kg fat in all scale-up studies. The fat in cream was adjusted after inoculation with cheese whey medium to about 50-55 per cent, a concentration which gave optimum lipolysis. It was essential to ensure that there was no separation of fat in cream during operations involving sterilization and subsequent incubation with the enzyme. A temperature of 90°C for 20 min was adequate for sterilisation of cream prepared under normal hygienic conditions.

The cream at the end of fermentation still showed about 20-25 per cent of the initial lipase activity. The stability of flavour in cream during storage was, therefore, a major problem because of further release of fatty acids due to the activity of residual lipase. A number of preservatives were tried in order to determine if the flavoured cream could be stored at ambient temperatures, but without much success (Table 2). Surprisingly, no free fatty acids could be detected within 4 days but off-flavours developed, accompanied by whey separation. There was extensive proteolysis which could be expected because of the presence of proteinases in culture medium[1] even when preservatives were added. There was also a marked reduction in yeast count in presence of preservatives such as benzoates and sorbic acid. Ideally, the cream could be preserved upto a month at 4°C without any change either in fatty acid content or composition.

The recovery of solids during preparation of the rennet curd is given in Table 3. As in any other cheese making process, upto 60 per cent of solids-non-fat (SNF) were lost during renneting operation in the form of cheese whey which could be reused as a growth medium to produce cell mass. Fat losses could also be avoided by using skim milk in place of 'standardised' milk (3.5 per cent

TABLE 2. FLAVOURED CREAM STABILITY DURING STORAGE

No.	Preservative	Storage Temp (°C)	Storage Time (days)	Yeasts cells (ml)	FFA (meq/ 100 g fat)	Flavour
1	Nil	25°C	0	2.75×10^5	26.02	Good
2	Nil	25°C	4	1.6×10^5	ND	Off-flavour, putrid
3	BHA+ BHT 0.1%)	25°C	4	2×10^5	ND	Off-flavour, whey separation
4	Sodium benzoate (0.1%)	25°C	4	7×10^3	ND	Off-flavour
5	Sorbic acid (0.1%)	25°C	4	1×10^2	ND	,,
6	Nil	4°C	3	1.28×10^5	31.60	Good flavour
			7	1.31×10^5	30.58	,,
			10	8.6×10^4	34.02	,,
			30	6×10^4	33.50	Good flavour, slight whey separation.

BHA = Butylated hydroxyanisole; BHT = Butylated hydroxytoluene; ND = Not detected.

TABLE 3. RECOVERY OF SOLIDS DURING PREPARATION OF RENNET CURD

Standardised milk					Rennet curd					Recovery	
Quantity (kg)	Fat (%)	Fat (kg)	SNF (%)	SNF (kg)	Quantity (kg)	Fat (%)	Fat (kg)	SNF (%)	SNF (kg)	Fat %	SNF %
500	1.95	9.75	7.71	38.5	45.0	19.18	8.62	38.40	17.30	88.50	44.9
1152	2.05	23.62	8.9	102.53	105.0	23.16	24.40	33.68	35.60	100.00	34.7
600	1.85	11.10	7.2	43.20	53.5	20.50	10.97	33.50	17.95	99.00	41.5
1000	2.25	22.50	7.90	79.00	92.0	23.50	21.65	31.88	31.20	96.00	39.5
500	2.05	10.25	8.70	43.50	52.0	21.00	10.90	33.00	17.16	100.00	39.4

fat). Milk curd, fresh and flavoured (fermented) creams were used for the preparation of the processed cheese. A typical composition of the ingredients for the melting operation is given in Table 4. All ingredients with the exception of the flavoured cream were heated in a Kustner melting kettle. The temperature was raised to 70°C within 2-3 min with rapid stirring. Flavoured cream was then added to the mix and the temperature of the kettle was further raised to 90°C. The mix was held at this temperature for about 15 min before packing hot in cans.

Comparative analyses of the processed cheese of some available brands in India are given in Table 5. Karnal cheese was an experimental sample kindly provided by Indian Dairy Research Institute and was more similar to the conventional cheddar cheese in terms of flavour and texture than other brands. Processed cheese manufactured by our process differed from other brands primarily in its lower content of acid-soluble nitrogen, acidity and total microbial count. Higher concentrations of acid-soluble nitrogen observed in other samples were probably due to the significantly higher degree of proteolysis by lactic cultures used in preparation of many of the conventionally ripened cheeses. Similarly, the higher bacterial count observed in these cheeses probably originated from the survivors of lactic starters used in the process. This problem was encountered to a much lesser degree in our process because of the relative sus-

TABLE 4. A TYPICAL COMPOSITION OF INGREDIENTS FOR MELTING OPERATION FOR PROCESSED CHEESE

Ingredients	Quantity (kg)	Fat (kg)	Other solids added (kg)	Water
Curd	15.630	3.440	—	7.190
Flavoured cream	3.330	2.000	—	1.330
Fresh cream	1.141	0.685	—	0.456
Water	3.649	—	—	3.649
SMP	0.250	—	0.250	—
Tripolyphosphates	0.125	—	0.125	—
Pyrophosphates	0.375	—	0.375	—
Sodium chloride	0.500	—	0.500	—
Sorbic acid	0.005	—	0.005	—
Colour	traces	—	traces	—
Total cheese	25.005	6.125	1.255	12.625

SMP = Skim milk powder

SNF in curd was 5 kg and total cheese was also 5 kg

TABLE 5. COMPARATIVE PROXIMATE COMPOSITION OF EXPERIMENTAL CHEESE AND SOME OF THE AVAILABLE BRANDS OF PROCESSED CHEESE

Analysis	Product brands				
	Indian		Australian canned	Karnal* canned	Experimental canned
	Canned	Cubes			
Moisture	44.53	44.34	44.37	41.92	46.37
Dry matter	55.47	55.16	55.63	58.08	53.13
Fat	25.60	27.20	25.86	27.70	23.31
SNF	29.87	27.96	29.77	30.38	29.32
Total protein	20.77	26.03	23.08	23.78	20.55
TCA-soluble nitrogen	0.57	0.32	0.32	0.56	0.14
Acidity**	2.05	1.77	2.11	2.18	1.54
pH	5.75	5.85	5.80	6.00	5.90
Microbial count/ml	2.3×10^5	1.5×10^4	500	5×10^3	500

*Control cheese sample provided by Indian Dairy Research Institute, Karnal.

**Acidity expressed as per cent lactic acid.

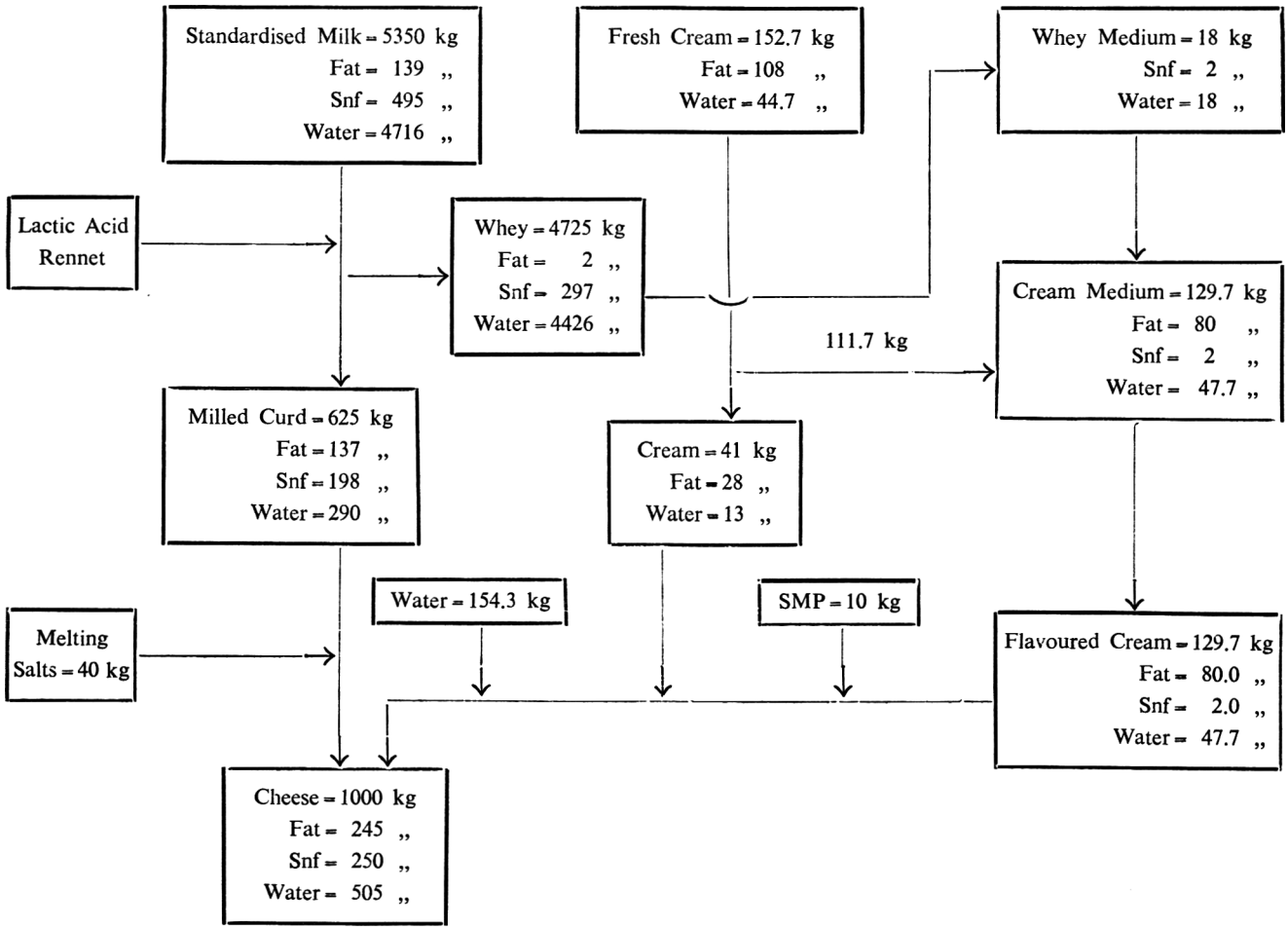

Fig. 2. Material balance for processed cheese using flavour

ceptibility of the yeast to the cheese melting temperatures. Material balance for the production of 1 tonne of processed cheese is summarised in Fig. 2.

Discussion

Many approaches have been used to reduce cheese ripening times, for instance, use of enzymes in cheese milk, raising temperature of ripening, increasing concentration of starters, and, even adding flavour cocktails.[6-10] More recently lipolysed milk products are available as flavour additives to bakery, confectionery as well as cheese spreads.[11] Each of these processes has its own limitations. Control of flavour development by use of enzymes during conventional ripening of the curd is not very easy and may often lead to the development of unacceptable flavours (rancidity) or bitterness.[10] Present commercial approach in the Western countries for the preparation of processed cheese is to use chemical flavour cocktails along with bland or partially ripened cheese curds. For India, however, most of these compounds have to be imported. The concept of using yeast strain to produce lipases as a part of overall process has the advantage in that it is relatively cheap since there are no cost-intensive purification steps involved, as in case of enzymes themselves. Furthermore, the flavour is produced separately and can be spray-dried or used as such for addition to a variety of dairy products such as cheese spreads, cheese powders, milk sweets, ghee, etc. as well as to non-dairy products. This concept can be extended further to develop other types of flavour compounds from lipolysed fat. Thus, the fatty acids formed in lipolysed cream can serve as precursors of additional flavour components such as lactones, methyl ketones, and secondary alcohols to produce the blue cheese flavour if incubated with spores of *Penicillium roquefortii*.[12] Though blue cheese itself is not acceptable in our country, it can potentiate other flavours when added in small amounts.

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A Packaging System for Storage and Transportation of Refined Groundnut Oil and Hydrogenated Oil

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Various alternative packaging systems for hydrogenated oil and refined groundnut oil were tried in order to find out a suitable substitute for the currently used 18 l. square tin which results in transit damage and consequent loss by leakage of the contents. Of these, both HDPE jerrycan and GI drums both of 20 l. capacity have been found to be suitable from the point of view of keeping quality of the oil and transport worthiness. However, lack of stacking facility and recycling ability disfavour the HDPE jerry can. The GI drums on the other hand has no such disadvantages and can be easily cleaned and recycled several times and its use works out to be more economical compared to the square tin when reused atleast three times or more.

In our country bulk packaging and transportation, of hydrogenated oil is being done in 18 l. square tins. The same container is being used for various other edible oils also and notably for refined groundnut oil. The 18 l. square tin is at present governed by an IS specification. Under normal trade practice the use of this container does not appear to have raised any serious problem. However, this is not so in the Defence Services which form the largest single organised consumer group and where goods have to be sent over long distances by rail and various means of road transport. It was reported that in case of hydrogenated oil the tin got damaged and there was considerable leakage loss of the contents especially in summer months. In 1974, when there was an acute shortage of hydrogenated oil, the Defence Services switched over to refined groundnut oil and the leakage losses were reported to be much higher. An investigation was therefore undertaken to find out an alternative packaging system which could be used more efficiently both for storage and transportation of hydrogenated oil and refined groundnut oil. The present paper gives an account of this work.

Materials and Methods

The different packaging systems studied are:

(*i*) 18 l. square tin conforming to IS: 916-1966[1] and some provided with a wooden crate holding only one filled tin. The crate conformed to the requirements of class 'A' of Defence Services Packaging Code[2] and was made using only parallel struts and fattens and no diagonal support.

(*ii*) 18 l. square tin, with two welded side seams and welded wire handle but otherwise conforming to the IS specification, and some provided with $\frac{1}{2}''$ thick coir rope packing all around. The ropes were tightly tied so that there were two rows of evenly spaced ropes parallel to each pair of opposite sides at each face.

(*iii*) Galvanised iron drums GI conforming to IS:2552[3], 20 l. capacity. These are made out of 24 gauge GI sheet, with side electrically welded and ends double interlocked and seamed. The drums are fitted with 10 cm neck with plugs and outer capseals and strong welded GI sheet handles. The dimension of the drums are 280 mm internal diameter $\times$ 380 mm overall height. At the side joint, due to high temperature welding, the galvanized surface is damaged. The outside damaged surface is repaired by solder coating but the inside surface at the weld joint, remains uncoated.

(*iv*) High density polyethylene jerry can of 20 l. capacity, rectangular in shape, fitted with a screw capped

opening and a centrally placed handle and finger grip at the bottom which enable easy tilting and emptying. The approximate dimensions are 30 cm × 31 cm × height 37 cm and a wall thickness of 2.5 mm to 2.75 mm. The jerry cans are understood to be made out of Hostalen GF 5260 grade of polymer manufactured by M/s Hoechst Dyes and Chemicals Ltd.

Hydrogenated oil: Hydrogenated oil conforming to ASC specification[4] was obtained from Army Supply Depot where it had already had a shelf life of 3 months.

Refined groundnut oil: This conforming to ASC specification[5] was also obtained from Army Supply Depot after 3 months' storage period. However, for vitamin 'A' estimation, fresh stock from a local mill without added vitamin 'A' was obtained.

Methods of Test:

Filling of containers: The square tins and HDPE jerry cans were filled with 16.5 and 18.5 kg respectively of the experimental samples having corresponding ullages of approximately 1.4 l. and 1l. In GI drums the filling was 18.5 kg mostly but in a few cases for vitamin A estimation it was 16.5 kg also. The ullages in these cases were 1.3 and 3.4 l. respectively.

1. *Drop test:* The finished packs were subjected to a free drop from a height of $2\frac{1}{2}$ ft using a drop tester and allowing the impact on the bottom face only. The dropping was repeated 4 times to see if the container was damaged and leakage occurred as a result of this damage.

2. *Accelerated storage test:* Packs of refined oil and hydrogenated oil in GI drum and HDPE jerrycan were stored at 37°C and then they were periodically taken out and examined for chemical and organoleptic tests.

3. *Transportation and storage in field areas:* Packs of refined oil and hydrogenated oil in both GI drums and HDPE jerry cans were sent without crating by rail transportation to Tezpur and Jodhpur from Mysore. The packs received at Tezpur were immediately sent back to Mysore where their condition and leakage loss, if any, were examined. The packs received at Jodhpur were stored under ambient condition upto 9 months and these were sent back to Mysore at intervals for chemical and organoleptic evaluation of the contents and for examination of the physical condition of the containers.

4. *Chemical tests:* Free fatty acid, iodine value and unsaponifiable matter were determined by AOAC methods.[6] Zn was estimated by the method of Sharma[7]. Peroxide value was determined by Wheeler iodometric method.[8] Vitamin A test was done by Carr Price antimony trichloride method.[9] Colour was measured by means of a lovibond tintometer using a 1 cm cell.

Refractive index at 40°C was measured using Abbe'y refractometer. In case of hydrogenated oil the value was converted to butyro refractometer reading.

5. *Organoleptic test:* Organoleptic acceptability was carried out on *puri* and *soji halwa* (sweat meat preparation) prepared by using the stored oils. A panel of 5 judges gave their ratings on a nine point hedonic scale.

Results and Discussion

A spot inspection of Army Storage Depot revealed that a good proportion of the tins suffered physical damage by way of heavy denting and distortions causing seam opening at the side and bottom joints and consequent leakage. The loss appeared to be quite appreciable though no authentic data could be obtained.

The type of damage suffered by some of the packs showed that they are liable to very rough handling. To test the physical soundness of a packaging system, therefore, it was necessary to prescribe a rather drastic test. In military transportation, there are different degrees of handling abuse at different stages. For example, it may be dropped from a railway wagon to the ground ($2\frac{1}{2}$ to $3\frac{1}{2}$ft), from a truck to the ground ($2\frac{1}{2}$ft), from mule back to the ground (2ft) or from a porters' hand to the ground (1ft). A $2\frac{1}{2}$ft drop was taken to be an average of these possibilities which a packaging system must be able to withstand to prove its physical soundness. Again there could be a minimum of 4 handlings of a pack if rail and road transportation is involved, hence the choice of the number of drops. However, if an experimental pack suffered heavy damage before the 4th drop, further dropiing was discontined. It has also been seen that a pack which survived 4th drop was capable of withstanding some more drops.

Initial attempts to tackle the problem either by improving the construction of the tin or by using crates or other outer packing proved futile as indicated by drop test (Table 1). Two of the readily available alternative containers, viz GI drum (20 l.) and high density polyethylene (HDPE) jerrycan (20 l.) were, therefore, subjected to drop tests and transportation trial (Table 1) and found suitable in this respect. Of these, the HDPE jerrycan was not considered to pose any toxicological problem as HDPE containers in capacity of 1 kg is already in commercial use for packing hydrogenated oil and the polymer is certified by its manufacturers to be free from any objectionable ingredients. Regarding the other container, though the GI drum is not approved at present by any known authority as a good container, it is in fact being used by many traders for transporting edible oils from factory to distribution centres where the contents are transferred to used 18 l.

TABLE 1. DROP TEST AND TRANSPORTATION TRIAL OF DIFFERENT PACKAGING SYSTEMS

Packaging container	Outer packing	Result of drop test	Transportation trial
18 l.sq tin as per IS:916-1966	Nil	Deformed bottom; bulged sides; after 1st drop bottom and side seam leaking	Trial not carried out
18 l.sq tin with two welded side seams	„	Deformed bottom; bulged sides; after the 1st or 2nd drop bottom seam leaking	„
18 l.sq tin with two welded side seams	½" coir rope	Less deformation, side bulging and denting; 2nd or 3rd drop, bottom and side seam leaking.	„
18 l.sq tin (No. 1)	Wooden crate	Crate damaged; deformation and side bulging; leakage after 2nd or 3rd drop	„
20 l.GI drum	„	No damage or leakage; very slight denting	Denting, no leakage in packs returned from Tezpur
20 l.HDP Jerrycan	„	No damage or leakage	No damage or leakage in packs returned from Jodhpur.

tins. It was considered necessary, therefore, to investigate the suitability of GI drum from the point of view of possible hazardous contamination which it could give rise to and also the keeping quality of the contents during storage over a long period. In the drums chosen for experimental use soldering was not done at any part which came in contact with the oils. The HDPE jerrycan was likewise used for ascertaining the quality deterioration if any, in the oils stored in it for a long period.

The results of storage studies of hydgrogenated oil and refined groundnut oil in the above types of containers under ambient conditions (Mysore and Jodhpur) or at 37° C are given in Tables 2 and 3. From these it can be concluded that both hygrogenated oil and refined groundnut oil do not suffer any marked colour change when stored in either of the two types of containers. The chemical changes are also negligible. Peroxidation in one case only was found to increase to 60 after 9 months' storage at Jodhpur. However, this occurred after more than 12 months' storage (including 3 months in Army depot when used for experimental purpose) and the oil was still acceptable. Both refined oil and hydrogenated oil remained acceptable in GI drum as well as HDPE jerry can during the above storage studies.

In GI drums, zinc contamination rose to a maximum of 0.95 ppm in case of refined groundnut oil and 4.15 ppm in case of hydrogenated oil. These levels are considered to be too low to pose any health hazards and are well within the limit of 50 ppm laid down for canned vegetables by ASC specification[10]. The oils were also tested for arsenic and lead but these were not traceable. From these tests therefore, it can be concluded that GI drums of the construction used does not pose any contamination hazards.

Army specifications lay down certain amount of vitamin A fortification of both hydrogenated oil and refined oil. To ascertain if the change in container will have any detrimental effect on the vitamin A content of the oils during storage, a comparative storage test under accelerated conditions (37°C) was carried out in both the 18 l. square tins and 20 l. GI drum. For this purpose, ASC supplies could not be used because of their uncertain initial concentration of vitamin A. Oil refined alone was used since this was available fresh from a local oil mill. Synthetic vitamin A was added and mixed thoroughly into the oil. The concentration at the beginning and after storage for 3 and 6 months in the two types of containers are shown in Table 4. It is observed that GI drum has no particular detrimental effect on vitamin A which is sensitive to oxygen *via* peroxide formation in oil. It is seen that with increased ullage in the container and consequently more of enclosed oxygen there is a corresponding higher loss of vitamin A.

Apart from suitability considerations of economy, convenience in handling and stacking are also important in the choice of the container. It is seen from the current rates that a 20 l. GI drum is three times as costly as a 18 l. square tin. A GI drum, however can be very easily cleaned with hot detergent solution and can be recycled several times. If used 3 times or more, it will work out to be more economical than the currently used 18 l. square tin. It is also convenient to handle and being structurally stronger can be stacked much higher than

TABLE 2. STORAGE STUDIES OF HYDROGENATED OIL AND REFINED GROUNDNUT OIL IN GI DRUMS

Storage temp (°C)	Storage period (months)	Colour yellow+blue	Refractive index at 40°C	FFA as oleic acid (%)	Peroxide value	Iodine value	Zn (ppm)
			Refined groundnut oil				
-2.5 to 44	Initial	0.5+0.3	1.462	0.07	8.29	94.1	0.38
,,	4-9	0.5+0.3	1.462-1.463	0.07-0.09	15.9-60.1†	92.5	0.90
37	Initial	0.5+0.3	—	0.09	2.62	95.2	0.64
,,	2-6	0.5+0.3 to 0.5+0.1	—	0.09-0.10	4.72-8.18	94.7	0.77-0.95
			Hydrogenated oil				
3-44	Initial	—	51.00*	0.06	3.30	—	0.92
	2-6	No visible change	52.0-52.9*	0.8-0.08	7.0 to 12.3	—	2.95
37	Initial	—	51.0*	0.24	3.10	—	0.92
	2-6	No visible change	51.95-52.90*	0.25-0.27	4.00 to 4.6	—	4.15

When refined groundnut oil used was received for studies, it had already a shelf life of 3 months in 18 l. tins. During the storage period at Jodhpur from March to November, the maximum and minimum temperatures were 44°C and -2.5°C respectively. Different batches were used for ambient storage at Jodhpur and for accelerated storage.

The hydrogenated oil used had already a shelf life of 5 months when received for studies. During the storage at Jodhpur from July to January the maximum and minimum temperatures were 44°C and 3°C respectively. Different batches were used for ambient storage at Jodhpur and for accelerated storage.

†Sample after 9 month's storage gave distinct rancid odour; however the oil was found acceptable when used in the manner described.

The results of 9 months' storage sample could not be given since the pack was received back at Mysore after a very long delay due to dislocation in transit.

All the samples were organoleptically acceptable even after the respective periods of storage.

Lead and arsenic contents were not traceable in any of the samples. After storage the unsaponifiable matter remained unchanged in refined groundnut oil (0.48%) and it was 1.20% in hydrogenated oil under both storage conditions.

*Butyro refractometer reading at 40°C.

TABLE 3. STORAGE STUDIES OF HYDROGENATED AND REFINED GROUNDNUT OILS IN HDPE JERRYCANS

Storage temp (°C)	Storage period (months)	Colour yellow+blue	Refractive index at 40°C	FFA as oleic acid (%)	Peroxide value
		Refined groundnut oil*			
18-32*	Initial	0.3+0.0	1.462	0.050	5.26
,,	12	0.4+0.0	1.462	0.070	12.95
37	Initial	0.3+0.0	1.463	0.050	5.26
,,	6	0.3+0.0	1.441	0.070	22.36
		Hydrogenated oil**			
3-44	Initial	—	51.00†	0.057	3.3
,,	6	No visible change	51.95†	0.146	9.1

*This was done from 2-month old sample.

**The samples used were 3-month old. The maximum and minimum temperature during the period (July-Jan) were 44°C and 3°C respectively,

All the samples were organoleptically acceptable.

†Butyro refractometer reading at 40°C.

TABLE 4. COMPARATIVE STABILITY OF VITAMIN A CONTENT OF REFINED GROUNDNUT OIL STORED IN 18 L TINS AND IN 20 L GI DRUMS

Container	Quantity of oil (l.)	Ullage	Storage period at 37°C (months)	Peroxide value	Vitamin A (IU/mg)
18 l.sq tin	18	1.4 l	Initial	5.37	24.0
	,,	,,	3	5.70	21.5
	,,	,,	6	6.76	19.2
20 l.GI drum	18	3.4 l	Initial	5.37	26.1
	,,	,,	3	7.70	23.4
	,,	,,	6	8.10	20.5
	20	1.3 l	Initial	5.37	19.2
	,,	,,	3	5.90	17.2
	,,	,,	6	6.50	15.0

the latter. A 20 l. HDPE jerrycan on the other hand is 2.5 times costlier than a square tin but being of plastic it will deteriorate in quality and hence it is not advisable to reuse it. It also cannot be stacked properly in godowns in its present form. Hence the use of HDPE jerrycan cannot be considered either economical or convenient.

In conclusion though GI drum can be recommended as an alternative bulk packaging for hydrogenated oil and refined oil its suitability for use for unrefined edible oils containing various degrees of moisture, mucilagenous matter and free fatty acids will need further investigation.

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The Enhanced Toxicity of Methyl Iodide to the Adults of *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) in the Presence of Carbon Dioxide

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The effect of carbon dioxide on the toxicity of methyl iodide to the adults of *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) was studied. When sub lethal doses of methyl iodide, i.e. 0.25 and 0.5 mg/l were used along with 10% CO_2, there was about 14 and 80% increase in mortality among *S. oryzae*. In the case of *T. castaneum* with the same percentage of CO_2, 0.5, 1.0 and 1.5 mg of methyl Iodide produced 20, 34 and 99% mortality. LD_{50} and LD_{95} values (%) for CO_2 alone for 24 hr treatment have also been determined for these two insects.

Methyl Iodide (MI), a liquid fumigant, is the most toxic among the methyl halides.[1] Because it has good fugacity and fungicidal property, coupled with its less destructive action (12 per cent) on methionine compared to methyl bromide (52 per cent) as reported by Majumder *et al.*[2] it has been recommended for inpackage fumigation of packed foods.[3] Since the cost of the chemical is quite high, techniques to enhance its toxicity require to be investigated. Carbon dioxide has been found to enhance the toxicity of fumigants like carbon disulfide, chloropicrin, ethylene dichloride, ethylene oxide[4], HCN, methyl bromide[5] and phosphine[6]. Experiments were, therefore, conducted to investigate whether CO_2 could potentiate MI toxicity in order that the dosage requirements of the latter could be brought down appreciably.

Materials and Methods

Test insects were two weeks old adult *S. oryzae* reared on sorghum and *T castaneum* reared on whole wheat flour with 5 per cent brewer's yeast at 25±1°C and 70±10 per cent R.H. Saturated concentration of MI was prepared according to the method of Muthu and Srinath[7]. *T. castaneum* (30) were kept in open petridishes and *S. oryzae* (30) in open-end glass tubes covered with cloth diaphragms secured by rubber bands. Four to five trials with three replicates each were carried out in 2.5l. desiccators for 24 hr at 26±2°C and 70±10 per cent R.H. A vacuum of 5.1 cm Hg (2 in. absolute) was created inside the desiccators and it was restored immediately after injecting CO_2. Only sub-lethal doses of MI and CO_2 were used in all cases, except for MI tested at 0.75 mg/l. on *S. oryzae*, where 20 per cent mortality was recorded (Fig. 1). At the end of fumigation the insects were transferred to vials containing food and were kept for 7 days at 25±1°C and 70±10 per cent R.H. The $LD_{50}$and LD_{95} values for CO_2 alone were calculated according to Finney[8]

Results

The LD_{50} and LD_{95} values (per cent CO_2) alone are given in Table 1. CO_2, by itself, was not toxic to *S. oryzae* upto 16 per cent and to *T. castaneum* upto 40 per cent. However, beyond this level, CO_2 is lethal to both the species. *T. castaneum* adults require about two times more CO_2 for fifty per cent and ninetyfive per cent mortalities than *S. oryzae*.

The enhanced toxicities of subiethal doses of MI in the presence of different percentages of CO_2 for *S. oryzae* and *T. castaneum* are given in Fig. 1 and 2 respectively. It may be seen from Fig. 1, that when 0.25 and 0.5 mg/l. MI were used along with 10 per cent CO_2, there was about 14 and 80 per cent increase in mortality among *S. oryzae*. About 20, 34 and 98 per cent mortality was noted respectively with 10 per cent CO_2 plus subiethal MI doses of 0.5, 1.0 and 1.5 mg/l.

Discussion

The data obtained in the current study prove that CO_2 can potentiate the toxicity of one more fumigant that

TABLE 1. LD_{50} AND LD_{95} VALUES FOR 24 HRS—CO_2 TREATMENT ON *S. ORYZAE* AND *T. CASTANEUM* ADULTS

Insect	Regression equation	LD_{50} (%)	LD_{95} (%)
S. oryzae	$y = 0.33 + 12.73x$	23.17	31.33
T. castaneum	$y = 2.81 + 11.23x$	49.66	66.07

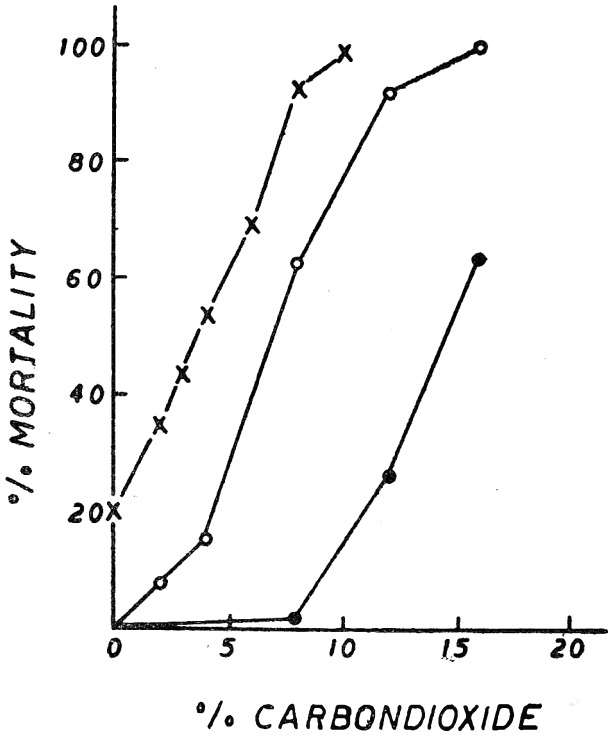

Fig. 2. Toxicity of MI to *S. oryzae* in the presence of CO_2. —×—, MI 0.75 mg/l; —O—, MI 0.50 mg/l; —●—, MI 0.25 mg/l.

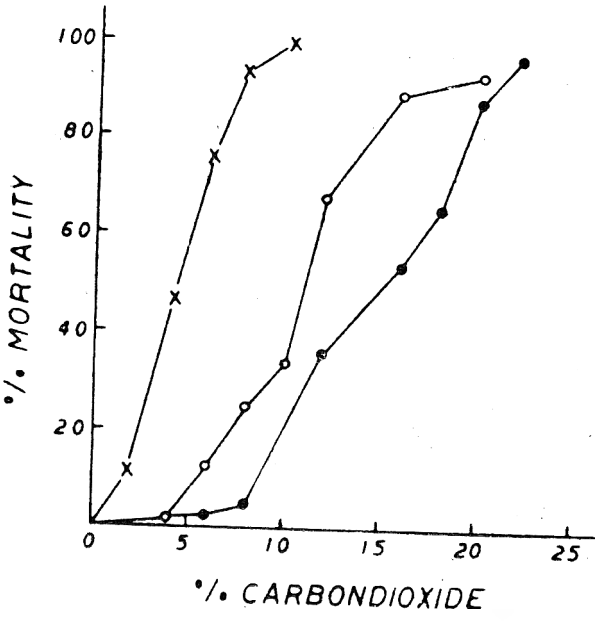

Fig. 2. Toxicity of MI to *T. castaneum* in the presence of CO_2. —×—, MI 1.5 mg/l; —O—, MI 1.0 mg/l; —●—, MI 0.5 mg/l.

is not yet reported. There seems to be a minimum lethal combination of MI and CO_2 to give a marked potentiation. For example, in *S. oryzae* at 0.25 mg/l. MI with upto 8 per cent CO_2, the increase in mortality was very low. Similarly, in the case of *T. castaneum*, at 0.5 and 1 mg/l. MI the potentiating action of CO_2 was negligible upto 4 and 8 per cent CO_2 respectively. Perhaps, these low percentages of CO_2 and MI doses are insufficient to bring about any physiological changes that would additively injure the insect. Beyond this threshold limit the insect may not be able to detoxify or eliminate MI in the presence of CO_2. Jones[9] reported that beyond certain percentage of CO_2, any further increase not only failed to potentiate the toxicity of ethylene oxide and methyl bromide but even decreased the effectiveness. Though in our studies, we did not come across such a situation, the maximum potentiation was observed at 12 and 8 per cent CO_2 with 0.5 and 0.75 mg/l MI against *S. oryzae* and 8 and 17 per cent CO_2 with 0.5 and 1 mg/l MI against *T. castaneum* respectively.

CO_2, though it acts as a respiratory stimulant at low levels, is lethal to insects when it exceeds the tolerance limit of the species tested. CO_2 is known to diffuse readily through animal tissues; as the partial pressure of CO_2 in the atmosphere is negligible, the elimination of of CO_2 in insects is by mere diffusion through the integument.[10] It may be possible to speculate here that when the atmospheric concentration of CO_2 is artificially increased not only the elimination of CO_2 from the insect body will be hindered but also the excess CO_2 outside may gain access into the insect body through the integumental membranes as well as through the 'permanently open' spiracles. CO_2 thus entered may prove lethal by affecting oxidative phosphorylation through a change in membrane permeability.[11]

In an atmosphere of MI-CO_2 mixture, the entry of the fumigant into the insect body may be greater as has been shown with phosphine.[6] The higher the percentage of CO_2, the greater will be the time lag for regaining normal respiration.[12] Consequently, lethal quantities of waste products will start accumulating and detoxification of the fumigant will be hampered. This may, perhaps, be the main reason for the additive effect of CO_2 and MI. Under such situations, even a sublethal dose of the fumigant which might under normal circumstances be detoxified and eliminated will suffice to produce a lethal effect. The present study gives promise that MI dosages could be reduced considerably by using appropriate percentages of CO_2 as the potentiating agent. Further work is in progress to adopt this finding for practical purposes so that MI could be made use of in large scale disinfestation programmes.

Acknowledgement

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Toxicity of Acrylonitrile—Ethylene Dibromide Mixtures to *Tribolium castaneum* Herbst and *Sitophilus oryzae* (L.) Adults

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Sublethal and $< LD_{50}$ doses of acrylonitrile (AN) and ethylene dibromide (EDB) were bioassayed alone and as a mixture at different proportions (w/w) against the adult stages of *Tribolium castaneum* Herbst and *Sitophilus oryzae* (L.) in void and in the presence of load (wheat) for 24 hr. Mortality was always higher with the AN-EDB mixture than when either was used alone. Comparison of mortality data arrived at the end of treatment and after 15 days showed variations for both the insects particularly with higher EDB doses; with *S. oryzae* the variation was unusually high.

No one fumigant is generally most toxic to all stages of all storage pests and no one fumigant has all the characterers of an ideal fumigant; hence, fumigant mixtures have been recommended in order that the desirable qualities of each could be exploited in fumigations.[1,2] Dilution of acrylonitrile (AN), a highly toxic fumigant, with nonflammable fumigants is obligatory as the former is explosive at 3.05 to 17.05 per cent by volume in air. AN has been successfully used with carbon-tetrachloride for fumigation of grains, packed cereal products, tobacco,[3,4] walnuts[5], potato,[6] with methylene chloride (or chloroform) for dry-wood termite control[4] and with tri-chloroacetonitrile as a household fumigant.[7] Bond and Buckland[8] have recently recommended AN-methyl bromide mixture for fumigations particularly at low temperatures. The results obtained in our preliminary tests with AN-EDB mixtures against the adult stages of the red flour beetle *Tribolium castaneum* Herbst and the rice weevil *Sitophilus oryzae* (L) in void and in the presence of wheat are presented here.

Materials and Methods

Two-to three-week old *T. castaneum* and *S. oryzae* adults reared on whole wheat flour plus 5 percent yeast and sorghum respectively at 30±1°C and 60±10 percent R.H. were used in all the experiments. The insects were contained in 6 cm × 2 cm glass tubes closed at both ends by cloth pieces tightly secured by small rubber rings. Fumigations were carried out in 0.85 l vacuum desiccators with septum caps (for injection of fumigant vapour/ liquid) at 30±1.5° C and 55±15 per cent R.H. for 24 hr in void as well as in the presence of load i.e., 300 g wheat of 10 per cent. moisture content which occupied approximately 47 per cent of the total volume of the desiccator.

For in void fumigations AN/EDB vapors from reservoirs containing saturated vapor concentrations of respective fumigants (doses calculated by using the formula: molecular wt.x vapour pressure/18.6)[9] were taken and administered using air tight glass syringes. For treatments in the presence of load, known quantities of AN/EDB liquids were injected over an evaporating surface of Whatman filter circle of 9 cm diameter. The dosages of AN and EDB were either sublethal or $< LD_{50}$ for the test insects. There were two replicates per (AN/EDB or AN-EDB mixtures) dose and the experiments were repeated 4 to 6 times. Mortality counts were made immediately after the treatment (only in experiments with load) and then transferred

to vials with food and kept at the rearing temperature and R.H. for 15 days after which final counts were made. Necessary corrections were made while arriving at final mortality data using Abbott's formula.[10]

Results and Discussion

Mean per cent mortalities obtained with different dosages of AN and EDB separately and as a mixture for the insects are given in Fig 1 and 2 and Table 1. In all the bioassay tests the AN-EDB mixture gave higher mortalities than that of either of the fumigant tested alone. In empty space fumigation high mortalities of *T. castaneum* were obtained (Fig 1) particularly with the mixture containing 0.6 and 0.8 mg/l of AN plus 1 and 1.25 mg/l of EDB. Below 0.6 mg/l or above 0.8 mg/l of AN plus EDB the potentiation was comparatively low. Above 0.8 mg/l of AN i.e., at 1 mg/l. dosage, AN itself gave 87 per cent mortality with *T. castaneum* (not shown in figure). With the above AN dose plus sublethal EDB doses of 0.25 and 0.5 mg/l the mortalities were 84 and 99 per cent respectively.

Mean mortalities of *T. castaneum* in the presence of load with 3 and 4 mg/l of AN and 1-4 mg/l of EDB alone and as a mixture are given in Fig 2. Mortality data at the post-fumigation period of 0 and 15 days reveals the appreciable differences in mortality wherever EDB doses were higher (3 and 4 mg/l of EDB doses). When AN was used alone the difference in the case of *T. castaneum* was however low. While EDB is known for delayed lethal action[11,12] the AN-EDB mixture has appreciable rapidity in exerting lethal effect as noted in mortality data at 0 hr. post fumigation day for *T. castaneum*.

TABLE 1. TOXICITY OF AN, EDB AND ANEDB MIXTURE TO *S. ORYZAE* ADULTS

Fumigant	Dose (mg/l)	Mortality (%)
No load		
AN	0.1	0
	0.2	11.7
	0.	
EDB	0.2	1.7
	0.4	18.2
	0.6	31.9
	0.8	46.7
AN+EDB	0.1+0.4	25.4
	0.1+0.6	74.8
	0.1+0.8	81.7
	0.2+0.2	13.0
	0.2+0.4	78.8
	0.2+0.6	70.4
	0.2+0.8	98.3
With load		
AN	1	0
	2	44.8
EDB	1	7.1
	2	40.6
AN+EDB	1+1	44.8
	1+2	76.3
	2+1	80.0
	2+2	94.5

Significant potentiation has been noted in void and with load treatments for *S. oryzae* also (Table 1). In fumigations with load per cent mortalities immediately after the treatment and after 15 days were wide apart.

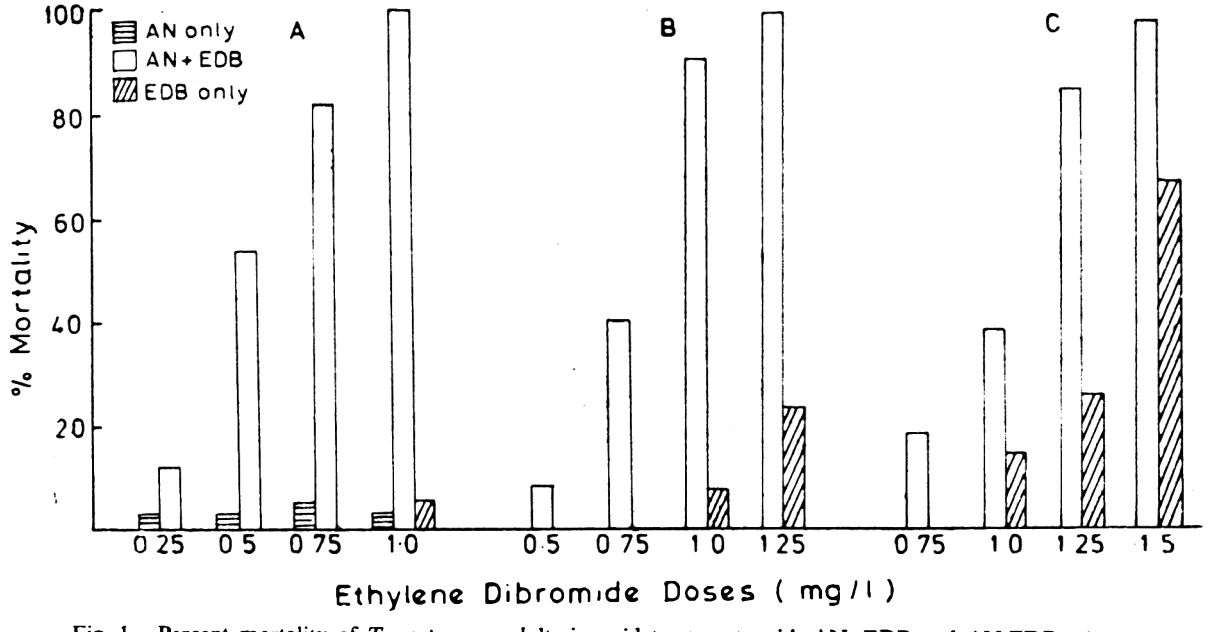

Fig. 1. Percent mortality of *T. castaneum* adults in void treatments with AN, EDB and AN-EDB mixture.
(A) AN 0.8 mg/l + EDB (B)AN 0.6 mg/l + EDB (C) AN 0.4 mg/l + EDB

TABLE 2. MORTALITY OF *S. ORYZAE* ADULTS IMMEDIATELY AFTER THE FUMIGATION (WITH LOAD)

Fumigant	Dose (mg/l)	Mortality (%)
AN	1	1.3
	2	5.7
EDB	1	1.3
	2	1.3
AN+EDB	1+1	1.3
	1+2	3.0
	2+1	8.7
	2+2	9.0

At O hr. post fumigation day the per cent mortalities at the relevent dosages are given in Table 2.

Examination of the insects immediately after the treatment did not show noticeable symptoms except that they were less active at higher dosages. In *T. castaneum* the difference in mortality at 0 and 15 days was observed with EDB alone or AN-EDB mixture treatments only; however with *S. oryzae* at all the dosages of AN, EDB and the mixture the difference was very high and the final mortality was unpredictable at O hr. post fumigation day. Such variations in mortality were also reported by Whitney and Herein[13] with carbon disulfide-carbon tetrachloride mixture and we also noted this when determining LD_{50} and LD_{95} values of AN[14] with *S. oryzae*.

AN (with carbon tetrachloride) and EDB (alone or admixed with other fumigants) are already in use as "spot fumigants". In mills and various food processing industries *Tribolium* spp. are the most common among the stored product insects. The above species are generally tolerant to AN[1,14]. Because of its very low vapour pressure EDB is persistent[15] and the problem of health hazards due to unaired fumigant residues is also there. The nonflammable AN-EDB mixture will be less persistent than EDB and is more toxic than either of the fumigants. The low dosages that are effective areanother attraction as problems of sorbed-residues are minimised.

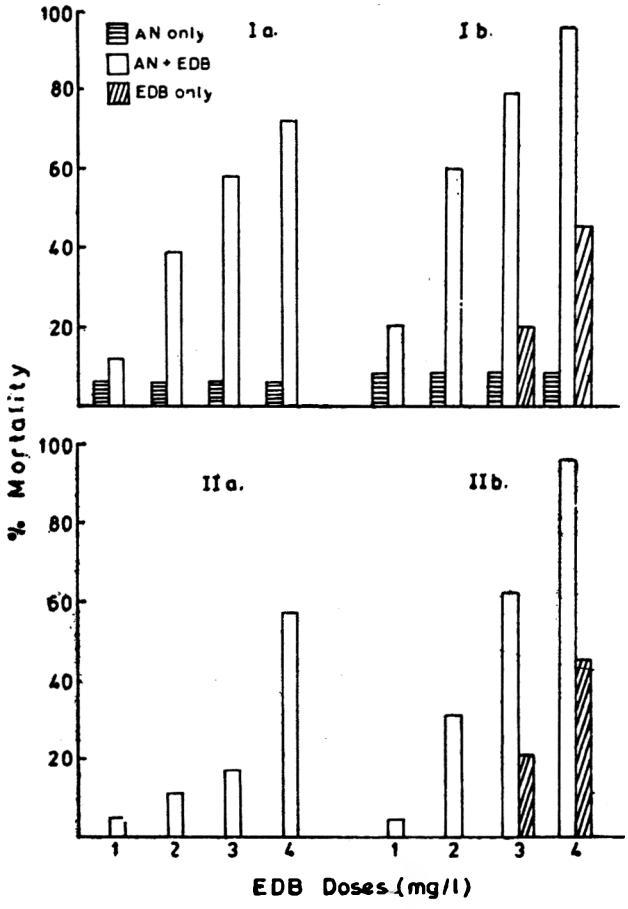

Fig. 2. Toxicity of AN, EDB and AN-EDB mixture to *T. castaneum* adults in the presence of wheat.

I. AN 4 mg/l. + EDB; (*a*) %mortality immediately after the treatment (*b*) %mortality after 15 days.

II. AN 3 mg/l. + EDB (*a*) %mortality immediately after the treatment (*b*) %mortality after 15 days.

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RESEARCH NOTES

OLIGOSACCHARIDE LEVELS OF PROCESSED LEGUMES

Raffinose, stachyose and verbascose content of cooked, germinated and cooked, and soaked legumes have been determined. In all the cases the levels of these oligosaccharides were found to be decreased by 45-80%, when estimated in the samples from which the water medium used for the processing was discarded.

Legumes have a predominant place in an average Indian diet. Though rich in lysine[1], their increased consumption causes flatulence, probably because of the oligosaccharides present in them[2]. In India, soaking, cooking as such or after germination and roasting are the common traditional processing practices followed in case of legumes before consumption. While studying the nutritive quality and flatulence causing ability of different legumes[3], an attempt was made to determine the effect of these common treatments on their oligosaccharide content.

Common legumes and pulses were therefore processed as follows. Red gram dhal (*Cajanus cajan*), Bengal gram dhal (*Cicer arietinum*), green gram dhal (*Phaseolus radiatus*) and lentil dhal (*Lens esculentus*) were bought from the local market and used for cooking. Each pulse (500 g) was cooked in 250 ml boiling water over a low flame for 30 min after which water was discarded. A known weight of horse gram (*Dolichos biflorus*) was soaked in water for 8–10 hr. The water was discarded and the seeds germinated for 24 hr at room temperature. Germinated seeds were then cooked for 30 min. over a low flame after which the cooking water was discarded. Black gram dhal (500 g) (*Phaseolus mungo*) were soaked in 500 ml water. Samples of dhal were removed at different time intervals. All the legumes treated as above were dried and the oligosaccharides extracted[4], separated[5] and estimated[6]. Standard verbascose was obtained as described previously.[7]

The oligosaccharide content of the raw pulses estimated here is in good agreement with those of Nigam and Giri[8]. As shown in Table, 1, on cooking for 30 min. in the presence of sufficient water and discarding the water, the levels of all the oligosaccharides changed substantially in the case of all the legumes studied. Loss of sucrose was the highest in Bengal gram amounting to 75.7 per cent followed by red gram with 67.2 per cent while both green gram and lentil had a more or less similar loss of 52.6 and 52.8 per cent respectively. However, the amount of this disaccharide is not of much importance as far as its effect on flatulence is concerned as it is easily digested. The loss in raffinose was the least in red gram being only 46.6 per cent as against 80 per cent in lentil followed by Bengal gram and green gram with 70.1 and 63.0 per cent respectively. On the other hand, red gram had the maximum loss of 80.7 per cent of stachyose, green gram had 75.8 per cent, lentil 68.5 per cent and Bengal gram 61 per cent. Loss of verbascose was maximum in bengal gram and in lentil being 72 per cent while green gram and red gram lost between 57-62 per cent. From all these observations it is clear that these oligosscharides are removed by the heat treatment used during cooking. The presence of these

TABLE 1. EFFECT OF DIFFERENT TREATMENTS ON OLIGOSACCHARIDE CONTENT OF LEGUMES (g%)

Legume		Sucrose	% loss	Raffinose	% loss	Stachyose	% loss	Verbascose	% loss
Red gram,	Raw	1.40	—	1.05	—	3.00	—	4.00	—
	Cooked	0.46	67.2	0.56	46.6	0.88	80.7	1.72	57.0
Bengal gram,	Raw	2.30	—	1.10	—	2.10	—	4.50	—
	Cooked	0.56	75.7	0.32	70.1	0.82	61.0	1.22	72.8
Green gram,	Raw	1.90	—	1.10	—	1.90	—	3.50	—
	Cooked	0.90	52.6	0.40	63.0	0.46	75.8	1.34	61.7
Lentil	Raw	2.50	—	1.00	—	2.60	—	3.10	—
	Cooked	1.18	52.8	0.20	80.0	0.82	68.5	0.84	72.9
Horse gram,	Raw	2.6	—	0.7	—	1.9	—	3.4	—
	Germinated 24 hr.	2.8	—	0.6	14.7	1.0	47.4	2.0	41.2
	Germinated & Cooked*	0.82	68.5	0.22	68.6	0.48	74.7	0.8	74.4

*Germinated and cooked for 30 min. in boiling water. Pulse to water ratio is 2:1.

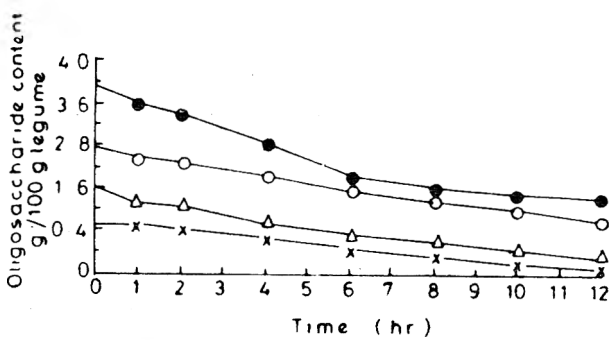

Fig. 1. Effect of soaking in water on th oligosaccharide content of black gram

— ● —, Verbascose; — O —, Stachyose; — △ —, Sucrose; — × —, Raffinose

and absence of their hydrolysis products in the discarded water[3] suggests that this loss is mainly due to their being leached out or solubilisation in the surrounding medium rather than due to hydrolysis or any breakdown. The extent of removal could therefore be influenced by the ratio of pulse to water and also the temperature of cooking.

Also as shown in Table 1 with horse gram on germination sucrose, increased by 7.7 per cent whereas raffinose, stachyose and verbascose decreased by 14.7, 47.4 and 41, 2 per cent respectively. The increase in sucrose may be due to the ∝-galactosidase action on galactosyl-sucroses[9,10]. Germination followed by cooking brought about a decrease in the levels of all oligoscacharides between 68 and 71 per cent which also resulted in decreased ability to produce flatulence in rats[3].

As shown in Fig. 1 over the total period of soaking of 12 hr the respective levels of sucrose, raffinose, stachyose and verbascose dropped from 1.6 to 0.28, 0.9 to 0.22, 2.3 to 1.1 and 3.5 to 1.4 g/100 g respectively of black gram dhal. The loss of verbascose and stachyase was more pronounced during the first 3-6 hr whereas raffinose levels decreased gradually from 0-12 hr. The loss of sucrose was the maximum being 82.5 per cent followed by raffinose (75.6 per cent) stachyose (52.2 per cent) and verbascose (60 per cent). However since in the present case no attempt was made to estimate the oligosaccharide content of the medium it is not clear whether the losses are due to leaching out or are absolute losses. Since, usually after soaking the dhal the water is discarded, it can be suggested that the soaking treatment which is one of the traditional ways of pre-processing the dhal can be recommended as a treatment for reducing the flatulence producing ability of the dhal at least to some extent as more than 50 per cent of all the 3 oligosaccharides supposed to be responsible for causing flatulence are lost by this treatment. Moreover continuous feeding of the legume treated in this manner also results in producing flatulence as low as 25 per cent of that produced with raw black gram dhal meal[3].

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EXTRACTION OF PROTEINS IN FRESH CHICKEN MUSCLE

Extractability of protein in red and white muscle of chicken was studied after ageing in ice. Extractability of protein increased after 4 hr ageing and remained almost the same even after 24 hr of ageing. The extractability of protein was higher in white muscle as compared to red muscle.

Post-rigor and tenderization of poultry meat is particularly rapid and extensive during the first few hours after slaughter.[1] Khan has reported extensively on the extraction and fractionation of proteins from fresh broiler chicken muscle with different buffer systems, viz. KCl-borate, KCl-phosphate, KCl-bicarbonate[2] and also under different storages above freezing temperature.[3-5] Weinberg and Rose[1] have reported the studies on protein extractability changes during post-rigor tenderization of chicken while muscle. The present study was intended to fix up the minimum time required to tenderize the muscle of culled chicken on ageing in crushed ice.

Chickens of 18-20 months old after fasting for about 20 hr. on the previous day were sacrificed by severance of the neck veins, bled, scalded at 60°C for 2-3 min.

TABLE 1. EXTRACTABILITY OF FRESH CHICKEN MUSCLE PROTEIN

Kind of muscle	SN as % TN after ageing in ice at indicated hr						NPN as % TN after ageing in ice at indicated hr					
	0	1	2	3	4	24	0	1	2	3	4	24
White muscle	71.7	69.7	70.1	68.5	80.2	79.0	13.7	14.7	14.4	14.7	14.1	14.0
Red muscle	52.6	47.6	48.2	48.0	63.1	64.3	9.0	9.9	9.3	9.5	10.3	9.6

Total Nitrogen: 3.515-3.746 for white muscle and 3.101-3.681 for red muscle

plucked and eviscerated by hands. The dressed chicken muscle was sampled (white and red muscle separately) in pre-regor condition and after ageing in ice; the dressed poultry was covered with slushed ice on an s.s. wire.

Chicken muscle was blended in Waring blender with chilled KCI-PO_4 buffer of pH 7.3 and T/2=0.55 for about 2 min. The extract was then centrifuged at 3000 rpm and an aliquot was used for nitrogen estimation. The non-protein nitrogen (NPN) of the extract was determined on a further aliquot after precipitation of the protein by trichloroacetic acid solution (5 per cent final concentration). All nitrogen estimations were carried out by Kjeldahl procedure.

It was observed in each case that protein extractability in white muscle is always higher than that in red muscle. The extraction of protein expressed as per cent total nitrogen in the muscles after 0, 1, 2 and 3 hr ageing in ice ranged from 68.5 to 71.7 for white muscle and from 47.6 to 52.6 for red muscle. A sudden increase in extractability was observed in muscle after 4 hr ageing in both kinds of muscles and the values ranged from 78.4 to 82.7 (a.v.80.2) for white muscle and from 58.2 to 68.7 (av. 63.1) for red muscle. The extractability values remained almost the same even after 24 hr of ageing ranging from 76.9 to 80.8 (av.79.0) in the case of white muscle and from 57.0 to 67.5 (av.64.3) in the case of red muscle. The fluctuation in the extractability of soluble protein, especially more pronounced in case of red muscle, is found to be due to bird to bird variation and large variation in fat content of red muscle. A very slight decrease in values of extractability at 4 hr and at 24 hr (i.e. about 2 per cent decrease) is due to the loss of water-soluble proteins through washing effect of ice.

The important observation that the sudden increase in protein solubility at 4hr ageing must be due to softening of muscle and the extractability remaining constant even after 24 hr ageing in ice, confirms that minimum of 4 hr ageing in ice is necessary to tenderize the muscle.

The NPN was 13-14 per cent of total nitrogen in white muscle and 9-10 per cent of total nitrogen in red muscle at all the periods of ageing of muscle. During ageing in ice even upto 24 hr the NPN fraction remained almost stationary. Slight fluctuation in NPN may be attributed partly to a variation from bird to bird as has also been observed in fish by Moorjani *et al.*[6]

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ENTEROTOXIGENIC STAPHYLOCOCCI IN RAW MEATS

Sixty six buffalo meat, 55 pork, 12 mutton and 7 chicken meat samples collected from Bareilly (U.P.) market were examined for enterotoxigenic *Staphylococci*. Ten per cent sodium chloride broth for enrichment followed by surface streaking on ETGPA medium of Baird-Parker was employed for isolation. Eighteen (27.27) percent) buffa.o meat materials, 14 (25.45) per cent) pork samples, 3 mutton samples and 1 chicken meat sample revealed coagulase positive *Staphylococci*. Five isolates from buffalo meat, 2 from pork and 1 from mutton were found to be enterotoxigenic. As many as 6 out of 8 enterotoxigenic isolates produced enterotoxin 'C' either alonge or in combination with others. Enterotoxigenic staphylococci from buffalo meat, pork and mutton are reported for the first time in this country.

Staphylococcal intoxication has been one of the leading food-borne illnesses even in developed countries. In India, where proper reporting of food-borne diseases is non existant, there have been a few scattered reparts[1-4] of staphylococcal food poisoning, all attributed to milk products. In order to assess the potential health hazard from meat borne staphylococcal intoxication,

TABLE 1. ENTEROTOXIGENICITY OF COAGULASE POSITIVE STAPHYLOCOCCI FROM DIFFERENT MEATS

Type of meat	Samples tested	No of samples having coagulase +ve *Staphylococci*	enterotoxigenic	Samples producing enterotoxin (s)				
				C	D	AC	CD	ACD
Buffalo meat	64	18	5	2	1	1	—	1
Pork	55	14*	2	1	—	—	1	—
Mutton	12	3	1	—	1	—	—	—
Chicken meat	7	1	nil	—	—	—	—	—
Total	138	36	8	3	2	1	1	1

*only 6 were tested for enterstoxigenicity

knowledge of the occurrence of enterotoxigenic *Staphylococci* in meats is essential. Prevalence of enterotoxigenic *Staphylococci* in meats is essential. Prevalence of enterotoxigenic *Staphylococci* in goat meat has been reported earlier[5] and their occurrence in buffalo meat, pork and mutton is reported here.

Sixty six buffalo meat, 55 pork, 12 mutton and 7 chicken meat samples, collected from the retail market in Bareilly (U.P) were examined for enterotoxigenic *Staphylococci.* Methods employed for isolation of the organisms and detection of enterotoxigenicity were the same as those reported in an earlier publication[5].

The results of examination of the meat samples are presented in Table 1.

In the present study, 27.27 per cent of buffalo meat samples and 25.45 per cent of pork samples were found to reveal coagulase positive *Staphylococci* while Gupta and Choudhury[6] recovered these organisms from 6.8 per cent of beef samples and 11.2 per cent of pork samples. The latter workers examined meat samples mostly from slaughter house whereas samples included in the present study were from retail meat market. This, amongst others, can be a factor responsible for higher percentage recovery of coagulase positive *Styphylococci* in the present study.

Five (27.27 per cent) out of eighteen coagulase positive isolates from buffalo meat, two out of six from pork and one out of three from mutton, were found to be enterotoxigenic. In an earlier study in respect of goat meat,[5] it was observed that as many as 7 (58.33 per cent) out of 12 coagulase positive isolates were enterotoxigenic. Thus it appears that a higher proportion of coagulase positive *Staphylococci* from goat meat are enterotoxigenic as compared to those, from other meats.

Four out of five enterotoxigenic isolates from buffalo meat and both the isolates from pork samples were found to produce enterotoxin 'C'. The only enterotoxigenic isolate from mutton elaborated enterotoxin 'D' Two out of the five isolates from buffalo meat and one out of two from mutton produced more than one type of enterotoxin while none of the seven isolates from goat meat were reported[5] to produce more than one toxin. Preponderance of enterotoxin 'C' producing isolates, observed in the present study, was also noted in respect of isolates from goat meat,[5] bovine milk[7] and human nasal carriers[7] in this country. This is also interesting in view of the reported predominance of enterotoxin 'D' producing isolates in frozen foods in U.S.A., by Casman *et, al.*[8] and enterotoxin 'A' producing strains in meat products in U.K. recorded by Simkovicova and Gilbert[9] and Payne and wood[10].

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PARBOILING OF SOAKED PADDY BY CLOSED HEATING

Soaked paddy could be parboiled by simply heating in a closed jacketed rotating drum to about 100°C. This eliminates the need of a boiler and the product obtained also will be having 4-8% less moisture content than normal steam parboiled paddy.

In the existing system of parboiling soaked paddy is steamed (usually from a boiler). It was thought of interest to study whether soaked paddy could be parboiled simply by heating in a closed atmosphere instead of steaming.

The experiments were conducted at the Modern Rice Mill in Sembanarkoil using the bran stabiliser designed by the Central Food Technological Research Institute. IR-20 paddy (7 months old) was used. Paddy soaked in the industrial parboiling tanks (capacity $3\frac{1}{2}$ tonnes each) of the Modern Rice Mill was used for the experiment, being soaked at 65°C for 4 hr. All experiments at a given temperature were carried out in a single day with the same batch of soaked paddy.

The bran stabiliser unit is a horizontal cylindrical drum with a holding capacity of about 320 kg of soaked paddy and fitted with baffles for efficient tumbling and mixing of the material. The drum is provided with a steam jacket and rotates around its horizontal axis at 9 rpm. It is also provided with pressure gauges in the steam jacket as well as in the inner chamber, a dial thermometer to indicate the temperature in the chamber and an air vent.

About 300 kg of soaked paddy, after draining, was charged into the drum. The lid was closed and steam was introduced into the jacket, the drum being rotated. The vent was kept open at this time to allow the air to escape. Steam supply was cut off when the desired temperature was reached and the air vent was closed. The drum was continued to be rotated for a further period as desired. The paddy was then discharged. It was immediately aerated in some cases and kept in a heap for 10 min in some other cases.

The paddy was subsequently shade dried and the milling quality was assessed by shelling in a 'Satake' laboratory husker and polishing in a 'Mc Gill' Mill No. 3 to approximately 5 per cent degree of milling. The polished rice was analysed for colour (visual), brokens, white belley, etc. Moisture was estimated by drying in an oven at 105°C till constant weight.

For comparison, a portion of the same soaked paddy was open steamed in the laboratory in a perforated aluminium steamer (*Idli* vessel) for 10 min. Drying, milling and analysis of rice were done as above. The treatments and the results are given in Table 1.

Milling tests showed that rice obtained from experiment 1 gave about 14 per cent of the kernels with white belley, showing that this temperature was insufficient for complete parboiling under the conditions tested. There was no white belley in the other samples and the milled rice appeared uniformly parboiled. The samples were all subjected to approximately 5 per cent degree of milling and the breakage in all cases was in the range of 0.5-2 per cent (and hence not shown). The control samples parboiled in the usual way (soaked paddy steamed) and shade dried also gave similar results. This showed that the above samples were effectively parboiled by closed heating. Heaping following heating helped somewhat at lower temperatures but was unnecessary at 95°C and above. Visually all the samples appeared nearly alike in colour, including the usual steam parboiled samples.

An important point is that while the steam parboiled paddy showed 3-5 per cent increase in moisture content after steaming, the closed heated samples, on the contrary, showed a decrease in moisture content in the

TABLE 1. TREATMENTS AND RESULTS OF PARBOILING BY CLOSED HEATING OF SOAKED PADDY

Expt. No.	Steam pressure in jacket (kg/cm^2)	Temp. at which steam cut of* (°C)	Time taken to reach the temp. (min.)	Duration of further heating (min.)	Pressure in the inner chamber (kg/cm^2)	Temp. of paddy on discharge (°C)	Time of heaping (min.)
1	5.0	85	40	10	0.2	95	10
2	3.0	95	75	5	0.0**	100	0
3	4.0	95	65	5	0.0**	100	10
4	4.0	95	65	5	0.2	97	0
5	5.0	95	50	5	0.2	100	10
6	4.0	100	110	5	0.0**	100	0
7	4.5	100	77	5	0.3	100	0

*Air vent was closed at this time except in Expts. 2, 3 and 6.

**Air vent kept open throughout heating.

range of 1-3 per cent (not shown). This will mean a reduction in the drying cost.

Prospects: The possibility of parboiling soaked paddy by closed heating without the use of steam has thus been established. This has several potentialities. Firstly if heating by steam in the jacket is also substituted by flue gases from a simple husk furnace, the need of a boiler will be totally eliminated enabling even small mills to undertake parboiling. Secondly if the drum is made sufficiently small, it can even be rotated by hand, further simplifying the process. Thirdly it is possible that in this method even paddy not fully soaked (say containing 25 per cent moisture) or even high moisture fresh paddy after harvest without presoaking, can be parboiled because of the diffusive effect of heating. This will result in further simplification of the process and further reduction in drying cost. Fourthly it is possible that by applying vacuum immediately after steaming the moisture content of paddy can be brought down in the drum itself perhaps to 18-20 per cent or so. These possibilities open up new prospects of a simple method of parboiling perhaps even at the farm level.

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WINE YEASTS AND THEIR FERMENTATION PRODUCTS

The capacity of four wine yeast strains for production of esters, aldehydes and higher alcohols during the preparation of wine and rate of utilization of sugar by these strains are reported in this communication.

It was believed that the aroma production in alcoholic beverages largely depended upon the nature of the raw material. With the increased knowledge about alcoholic fermentation, greater importance is being stressed on the role of yeast in the production of esters and higher alcohols, which contribute to the development of aroma and flavour. Although these constituents are produced by all yeasts, depending upon the strain of yeast, a considerable variation in the formation of isobutanol, isoamyl and active amyl alcohol and a proposal has been noticed during wine production.[1]

TABLE 1. FALL IN BRIX (HYDROMETER) AND ETHYL ALCOHOL FORMED DURING FERMENTATION

Time hr	Yeast Culture No.			
	101	374	379	801
	Brix°			
0	16.500	16.500	16.500	16.500
24	10.000	11.000	11.500	11.500
48	5.250	5.750	6.250	6.100
72	0.941	0.997	1.000	0.990
	Ethyl alcohol (%)			
24	1.20	1.20	1.00	0.90
48	2.15	2.22	2.22	2.15
72	7.82	7.80	7.72	7.82

Saccharomyces cerevisiae var. ellipsoideus—No. 101, 374, 379 and 801 maintained in the culture collection of CFTRI were used.

Estimation of alcohol, esters, aldehydes, volatile acid and higher alcohols were carried out according to the standard procedure of A.O.A.C.[2] Tannin was estimated by Folin and Dennis[3] method. Sugar was estimated by Somogyi[4] method.

Sulphited grape juice (*Vitis labrusca*) of 16.5° Brix° reducing sugar, 15.7 per cent, tannins, 93.5 mg per cent and acidity, 0.75 per cent (as tartaric) was used for fermentation studies. Starter culture prepared by inoculating the above cultures of yeast in sterilised grape juice, were added to bulk grape juice and fermented at temperatures ranging from 24 to 28°C for 72 hr and analysed every 24 hr interval.

It is seen from Table 1 that the fall in Brix° indicating the rate of sugar utilization picked up at the end of 24 hr and continues upto 72 hr which corresponds to the logarithmic growth phase of yeast. At the end of 72 hr hardly about 1 per cent sugar was left unfermented. The rate of formation and total quantity of ethyl alcohol produced was practically the same by all cultures (Table 2).

It is seen from table 2 that Culture No. 374 produced the highest level of esters (387.2 ppm) and aldehydes

TABLE 2. ANALYSIS OF THE WINE AT THE END OF 72 HOURS

Culture No.	Volatile acidity as acetic %	Esters as ethyl acetate ppm	Aldehyde as acetaldehyde ppm	Higher alcohols as isoamyl & isobutyl ppm
101	0.0102	293.2	110	240
374	0.0048	387.2	137	272
379	0.0036	281.6	110	304
801	0.0156	246.3	82	216

(137 ppm), culture 801 produced the highest level of volatile acid (0.0156 per cent) and culture 379 produced the highest level of higher alcohol (304 ppm). It is only in culture 101 that all these constituents were produced in moderate levels.

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STABILITY OF BACTERIAL RENNET AND OTHER MILK CLOTTING ENZYMES UNDER DIFFERENT STORAGE CONDITIONS

The present report refers to the effect of storage, in solid and liquid form, at deep-freeze, refrigeration and room temperatures on the milk clotting and proteolytic activities of bacterial rennet, Meito rennet, Hansen's rennet, pepsin and the combination of bacterial rennet with the other three milk clotting enzymes, No appreciable loss in milk clotting activity was observed in any of the single or mixed coagulants upto a period of 7 days on storage at deep-freeze temperature either in solid or liquid form. Loss in activity on subsequent storage was comparatively more in liquid form, on storage at refrigeration temperature in solid form, the loss in milk clotting activity was negligible in all the coagulants upto three months. The enzyme solutions were, however, stable only upto 15 days storage at this temperature, with the only exception of bacterial rennet which, though stable upto 24 hr, showed gradual loss in activity on subsequent storage. The effect of room temperature on the milk clotting activity was more drastic especially when the coagulants were stored in liquid form. While effect of storage on the proteolytic activity of various coagulants was not as severe as on the milk clotting activity, it followed more or less the same general pattern.

The enzyme rennet is almost invariably employed for the production of different varieties of cheese, although pepsin under the trade name of metroclot[1], plant rennet[2] and microbial rennet[3,4] have also been used as substitutes for the calf rennet. In recent years, mixture of calf rennet and pepsin[5-7] or calf rennet and microbial rennet[8-10] have also been successfully used in cheese making Whereas considerable attention has been devoted on various aspects of fungal rennet elsewhere, and on the development, production and purification of bacterial rennet from *Bacillus subtilis* K-26 in our laboratory[11], not much data is available on the stability of these preparations singly or as mixed coagulants under different storage conditions. The present report deals with the storage stability of bacterial rennet (*B. subtilis* K-26), singly and in combination with and in comparison to, calf rennet and other rennet substitutes at different temperatures.

The four different milk clotting enzymes used in this study were crude bacterial rennet (*Bacillus subtilis* K-26) from our laboratory, Meito rennet (*Mucor pusillus* Lindt.), Hansen's rennet (Chr. Hansen's Laboratory, Denmark), and bovine pepsin. Besides the four milk clotting enzymes used singly, bacterial rennet was mixed with Meito rennet (50:50), Hansen's rennet (50:50) and pepsin (75:25). All the seven coagulants (four singles and three mixtures) were stored both as solid and as 5 per cent stock solution at deep freeze temperature (-12 to -18°C), refrigeration temperature (4 to 6°C) and at room temperature (30 to 40°C) for 0, 1, 7, 15, 30, 60 and 90 days. The solid and the liquid preparations were made upto 1 per cent solution in distilled water before assaying for milk clotting and proteolytic activities at the end of each storage period. The milk clotting activity was assayed in triplicate according to the method of Srinivasan *et al.*[12] except that reconstituted skim milk (12 per cent) fortified with 0.01M calcium chloride was used as substrate. The proteolytic activity was determined in duplicate using a 1 per cent freshly prepared casein solution (pH 6.0) as the substrate. To 2.0 ml of the substrate, tempered at 30°C for 5 min, 4.0 ml of the coagulant solution was added and after mixing incubated at 30°C for exactly 20 min. Subsequently 4.0 ml of 12 per cent trichloroacetic acid was added to the reaction mixture to terminate the reaction. The precipitated proteins were then filtered through Whatman No. 1 filter paper and free tyrosine in the filtrate determined. For tyrosine determination, 4.0 ml of alkaline copper sulfate solution was added to 2.0 ml of the filtrate and mixed well. After 10 min, 0.5 ml of 1:1 diluted Folin Ciocalteu reagent was added to the tubes, mixed again and allowed to stand for 30 min. Intensity of the blue color developed was measured at 640 nm in a Elico Spectrocal Colorimeter and the optical density values were converted to milligrams of tyrosine from the standard curve of tyrosine prepared in a similar manner[13,14].

Initially, Meito rennet exhibited the maximum milk clotting activity followed by pepsin, bacterial rennet and Hansen's rennet. Amongst the mixed coagulants also, Meito rennet in combination with bacterial rennet showed better activity than the other two combinations. The effect of storage conditions on the milk clotting activity of the various coagulants is shown in Table 1. When the storage study was carried out at deep-freeze temperature, there was almost no loss in activity upto a period

TABLE 1. EFFECT OF STORAGE ON MILK CLOTTING ACTIVITY*

Storage		Single coagulants				Mixed coagulants		
Temp.	Period	Bacterial	Meito	Hansen's	Pepsin	Bact+Meito	Bact+Hansen's	Bact+Pepsin
				Solid storage				
Deep-freeze	0	100	100	100	100	100	100	100
	30	86.1	100	100	85.7	100	98.2	97.1
	90	84.7	99.6	76.8	82.3	99.5	95.3	95.9
Refrigeration	0	100	100	100	100	100	100	100
	30	99.1	100	99.8	96.8	100	100	96.5
	90	92.0	100	90.5	94.1	99.5	91.2	93.5
Room Temp.	0	100	100	100	100	100	100	100
	1	90.3	97.0	90.9	90.0	95.2	82.4	86.5
	15	67.9	87.8	68.0	88.6	91.3	55.3	56.3
	30	48.9	87.5	44.4	75.8	73.9	50.0	51.8
				Liquid storage				
Deep-freeze	0	100	100	100	100	100	100	100
	30	77.1	98.9	100	88.1	100	99.4	90.6
	90	77.6	97.7	56.6	79.9	92.3	66.5	85.3
Refrigeration	0	100	100	100	100	100	100	100
	30	73.3	88.5	73.4	83.1	92.8	88.8	93.5
	90	14.8	59.1	15.8	60.7	27.3	15.9	51.5
Room Temp.	0	100	100	100	100	100	100	100
	1	70.1	79.5	75.5	60.0	85.0	68.8	35.9
	15	8.1	57.5	14.8	46.8	22.5	8.3	18.8
	30	7.3	35.1	36.4	37.5	14.5	6.2	14.9

*Activity expressed as percent of the initial activity.

of 7 days in any of the coagulants under examination either in solid or liquid form. On further storage in solid form, while, Meito rennet was stable upto a period of 90 days, Hansen's rennet maintained the initial activity upto 30 days. All the mixed coagulants were stable upto three months. In liquid storage also, Meito rennet singly and in combination with bacterial rennet lost only 2.3 per cent and 7.7 per cent, respectively, of this initial activity at this end of three months. The better stability of mixed coagulants has been attributed to the synergistic effect of the component enzymes.[9] Stability of the enzymes at refrigeration temperature was found to be better than at deep-freeze temperature. While all the preparations stored in solid form were quite stable upto three months, decline in activity in the case of liquid storage was observed after 24 hr. However, Meito rennet singly had retained 88.5 per cent and in combination with bacterial rennet 92.8 per cent of the initial activity upto 30 days storage period. Likewise, pepsin alone and in combination with bacterial rennet was quite stable upto one month, maintaining 83.1 and 93.5 per cent of the initial activity, respectively. At the end of three months storage, pepsin and Meito rennet singly and pepsin in combination with bacterial rennet were found to be the most stable coagulants. Thus, it may be inferred that solutions of all the coagulants can be safely stored in the refrigerator for 24 hr. Further, with the only exception of bacterial rennet which gradually loses activity after one day, all the enzyme preparations singly and in combination with bacterial

TABLE 2. EFFECT OF STORAGE ON PROTEOLYTIC ACTIVITY*

Storage		Single coagulants				Mixed coagulants		
Temp.	Period	Bacterial	Meito	Hansen's	Pepsin	Bact+Meito	Bact+Hansen's	Bact+Pepsin
				Solid storage				
Deep-freeze	0	100	100	100	100	100	100	100
	30	89.2	100	81.8	100	95.4	95.1	91.2
	90	78.5	95.9	68.2	94.3	95.4	92.1	91.2
Refrigeration								
	0	100	100	100	100	100	100	100
	30	100	100	100	94.3	95.4	100	95.6
	90	100	88.5	90.9	94.3	91.8	92.1	95.6
Room temp.	0	100	100	100	100	100	100	100
	1	100	100	100	100	100	92.1	88.5
	15	93.3	95.6	100	94.3	95.4	84.0	59.0
	30	82.2	91.2	100	94.3	81.9	76.2	59.0
				Liquid storage				
Deep-freeze	0	100	100	100	100	100	100	100
	30	82.2	95.6	90.9	94.3	95.4	92.1	91.2
	90	78.5	91.2	68.2	78.6	86.6	88.0	69.3
Refrigeration	0	100	100	100	100	100	100	100
	30	68.0	90.0	36.4	94.3	95.4	81.5	73.9
	90	40.0	90.0	27.3	78.6	72.5	57.1	69.3
Room temp.	0	100	100	100	100	100	100	100
	1	89.2	100	60.0	94.3	100	67.9	95.6
	15	82.2	95.6	45.5	64.3	77.3	88.3	48.7
	30	68.0	91.2	36.4	64.3	72.5	81.5	44.2

*Activity expressed as percent of the initial activity.

rennet may be stored at refrigeration temperature upto 15 days with only 2 to 11 per cent loss in activity. However, storage at room temperature, even in solid form, caused a considerable loss in milk clotting activity which ranged from 3 to 17.6 per cent after 24 hr. storage. Meito rennet alone and in combination with bacterial rennet were found to be the most stable preparations losing, only 12.5 and 26.1 per cent respectively of the original activity after one month storage. Amongst the other coagulants, pepsin was quite stable, retaining 88.6 per cent of the original activity after 15 days and 75.8 per cent after 30 days. The effect of room temperature was more drastic when the enzyme preparations were stored in liquid form. A total loss in activity was observed in the case of bacterial rennet, Hansen's rennet singly and in combination with bacterial rennet, and the bacterial rennet pepsin mixture after a period of 60 days storage.

The initial proteolytic activity of bacterial rennet was found to be the highest amongst all the single and mixed coagulants evaluated. Microbial rennets, in general, have been shown to exhibit more proteolytic activity than either animal rennet or pepsin[15,16]. On storage, the proteolytic activity of the different milk clotting enzymes was not as severely affected as the milk clotting activity. This may be indicated from the results presented in Table 2. At deep-freeze temperature, the proteolytic activity of all the coagulants, with the exception of bacterial and Hansen's rennet, was not affected significantly even after a period of three months storage in

solid form. In liquid storage at the same temperature, the activity profile was more or less the same as observed in the case of solid storage. While maximum loss in activity was found in Hansen's rennet (31.8 per cent) among the single coagulants, 30.7 per cent loss in activity was exhibited by the mixed coagulant bacterial rennet and pepsin at the end of 90 days. At refrigeration temperature, the loss in activity in all the solid preparations was only marginal when evaluated after three months. However, when the storage study was carried out at this temperature in liquid form, a significant and gradual loss in activity was noticed with the duration of storage. While maximum loss in activity was observed in Hansen's rennet (72.7 per cent), Meito rennet singly had retained 90 per cent and in combination with bacterial rennet 72.5 per cent of the original activity at the end of three months. At room temperature also, the loss in activity was only marginal upto 15 days storage in solid form for all the enzymes except the two mixed coagulants bacterial and a Hansen's rennet, and bacterial rennet and pepsin. At the end of 30 days, while Hansen's rennet retained the original activity, loss in activity ranged from 5.7 to 41 per cent for the other coagulants. The adverse effect of storage at room temperature was somewhat more pronounced when the enzyme samples were stored in liquid form, though not as drastic as observed on the milk clotting activity.

The initial ratio of milk clotting to proteolytic activity varied from 0.009, for bacterial rennet singly and in combination with Hansen's rennet to 0.026 for pepsin. While there was hardly any change in these values in a particular coagulant on storage at deep-freeze temperature, either in solid or liquid form, and at refrigeration temperature in solid form, the ratios changed rather significantly in most cases when the enzyme preparations were stored in liquid form at refrigeration temperature and at room temperature in either mode of storage. This was apparently due to milk clotting activity being affected more in comparison to the proteolytic activity.

The present report, though limited in scope, provides some pertinent and useful data on the behaviour of bacterial rennet (*B. subtilis* K-26) and other milk clotting enzymes, singly and in combination with bacterial rennet, on storage under the specified conditions of the study.

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PROTEIN CONTENT AND AMINO ACID COMPOSITION OF PEARL MILLET

Grains of eight reconstituted pearl millet (*Pennisetum typhoides* Linn. (Brum) Stapf & Hubb.) hybrids were examined for total protein content and amino acid spectra. Protein values ranged from 11.0 to 14.7% lysine and threonine, which are limiting amino acids according to chemical score, showed a range of 2.56-3.46 and 1.99-2.44 (g/16g N), respectively.

Pearl millet (bajra) forms the staple diet of millions of people in India and some regions of Africa. These are consumed as unleavened bread (*chapati*) along with one of the grain legumes. However, very few studies to assess their nutritional quality characteristics have been conducted[1-5].

We have earlier reported the amino acid composition of various cereals and pulses consumed in India[6-8] In this communication, protein content and amino acid composition of eight reconstituted pearl millet hybrid grains released for commercial cultivation are reported.

Eight high yielding reconstituted Pearl millet (*Pennisetum typhoides* Linn. (Brum) Stapf & Hubb.) hybrids *viz.* (1) White grain hybrid (2) Old HB 3 (23 A×J 104) (3) 5141 A×D-32 (4) 5141 A×J 104 (5) 5054 A×J 104 (6) Hyb. PHB-14 (L 111 A×PIB 228) (7) 5141 A×K 560 (1576) (8) Hyb D-111, grown at the Experimental Farm of Indian Agricultural Research Institute, New

TABLE 1. AMINO ACID COMPOSITION AND PROTEIN CONTENT OF THE GRAINS OF PEARL MILLET HYBRIDS

Amino acid	Amino acid content (g/16 g N) of the hybrids							
	1	2	3	4	5	6	7	8
Lysine	3.42	3.37	2.65	2.83	3.46	2.56	2.86	3.44
Histidine	2.37	2.13	1.84	2.17	3.13	2.21	2.34	2.33
Arginine	5.22	4.36	4.49	4.49	6.17	3.84	4.53	4.93
Aspartic acid	5.06	4.23	4.56	4.32	5.48	4.99	4.99	5.42
Threonine	2.35	2.33	1.99	2.16	2.44	2.16	2.34	2.20
Serine	3.81	3.28	3.04	3.45	4.39	3.90	3.98	3.93
Glutamic acid	14.34	12.06	11.62	12.88	14.04	14.59	14.68	14.79
Proline	5.64	5.37	5.13	3.90	5.36	4.47	4.40	4.60
Glycine	3.02	2.60	2.15	2.53	3.11	2.50	3.16	3.10
Alanine	7.80	6.69	6.54	6.59	7.88	7.20	7.23	7.49
Cystine	1.96	1.87	1.92	1.78	2.28	2.12	2.61	1.93
Valine	4.59	4.00	3.83	4.06	5.82	5.06	4.44	4.93
Methionine	2.05	1.54	1.35	1.73	2.59	2.02	1.99	1.85
Isoleucine	4.01	3.09	3.57	3.52	4.56	4.22	3.77	4.26
Leucine	8.79	7.62	8.42	8.09	8.86	9.22	8.69	9.61
Tyrosine	3.14	2.73	2.68	2.42	3.61	3.08	3.06	3.20
Phenylalanine	4.55	3.90	4.09	3.44	5.15	4.35	4.06	4.47
Protein %	11.8	11.0	14.3	12.7	12.3	14.7	14.6	13.7
Chemical score %	59	58	48	51	61	46	52	55
Limiting amino acid	Thr	Thr	Lys	Lys	Thr	Lys	Lys	Thr

*For corresponding names of hybrids see text.

Amino acid values are based on actual recoveries; Protein percentage values are presented on dry weight basis; Calculated according to Block and Mitchell[10] by using FAO Reference Pattern.[11]

Delhi, during the crop season 1975 with fertilizer application of NPK (40:40:40 Kg/ha), were utilized for this study.

Representative samples were ground to 60 mesh size in Wiley mill. Nitrogen was determined using Technicon Autoanalyser[9] and the protein values were computed by using a factor of 6.25.

Amino acid analysis was done on Technicon Sequential Multisample Amino Acid Analyser. Hydrolysis procedure adopted was as described earlier[6,7].

Protein content varied from 11.0 to 14.7 per cent. Uprety and Austin[1] reported a range of 11.3 to 19.6 in some of the millet grains tested. Burton *et al*[2]. observed a variation from 8.8 to 20.9 per cent in the 180 inbred lines examined.

Amino acid spectra of the grains revealed that lysine content varied from 2.56 to 3.46 (g/16 g N). Theronine content had a range of 1.99-2.44 (g/16 g N). On the basis of chemical score these two amino acids are the most limiting. Glutamic acid content was lower than that of cereals reported earlier.[7] High leucine/isoleucine ratio has been reported to be causative factor for incidence of pellagra disease[12,13]. This is particularly with reference to maize and sorghum which have a ratio of 3.5-4.0: 1.0[7,14]. In millet grains the ratio is 1.9-2.5: 1.0. Glycine content, which varied from 2.15-3.16 (g/16 g N) was however, low as compared to that of other cereals. Its importance has been stressed in the feeding of chicks.[15]

The above amino acid spectral analysis clearly shows that protein quality of the hybrid millet grains is comparable to that of rice and oats.

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IMPROVEMENT IN THE FLAVOUR QUALITY OF (CARDAMOM) OIL FROM CULTIVATED MALABAR TYPE CARDAMOM GROWN IN KARNATAKA STATE

Removal of part of 1,8-cineole present in the oil of cardamom of cultivated Malabar type by fractional steam distillation considerably improves the quality of the oil comparable to the oil obtained from cardamom of cultivated Mysore type.

Cardamom is widely used as a spice in food and flavour industry. The cardamom grown in South India can be broadly classified into (*i*) cultivated Mysore type mainly grown in Kerala (Alleppey, Vandanmettu, etc.) and (*ii*) cultivated Malabar type mainly grown in Karnataka (Coorg, Saklespur, Sirsi, etc.) Both belong to the variety '*Elettaria Cardamomum* Maton Var *minuscula* Burkhill.[1-3] Recently, cardamom oil is also being produced and exported in substantial quantities. The oil obtained from cultivated Mysore type is favoured by the importing countries, whereas there is no demand for the oil obtained from cultivated Malabar type. So it was thought to devise a method by which the quality of the oil of the cultivated Malabar type could be improved, comparable to that of cultivated Mysore type.

There are excellent reports on the chemical compositions of cardamom oil of both types[1,4,5,7]. The major constituents of the oil are 1,8-cineole and α-terpenyl acetate. The cultivated Malabar type contains about 40 per cent cineole whereas the cultivated Mysore type contains only about 25 per cent of cineole. The pronounced camphoraceous odour of the cultivated Malabar type could be attributed to the presence of cineole in large amounts. To remove the excess cineole present in cultivated Malabar type, fractional steam distillation of the ground seeds of the cardamom was resorted to. Green cardamom capsules obtained from Coorg was air dried at 60°C for 9 hr. and dehusked. One kilogram of dried capsuke (moisture content 9 per cent) gave 720 g of seeds. Steam distillation was carried out in a special apparatus developed in this laboratory[6] where steam at slightly above atmospheric pressure was passed through a packed bed of ground cardamom seeds in an insulated cylindrical glass chamber and the distillate collected. Three batches of fractional steam distillations of ground cardamom seeds were carried out. The physical properties of the fractions, viz. refractive index, specific gravity and optical rotation were determined. The fractions were screened by GLC for their cineole and α-terpenyl acetate content. Cineole content of the second fractions were estimated by o-cresol method of Cocking.[8] The results are given in Table 1. A sample of oil of cardamom of cultivated Mysore type was obtained from this laboratory.

It is evident from the results in Table 1 that the second fraction of Batch 2 compares favourably with oil from cultivated Mysore type. The first fractions of Batches 2 and 3 contain major amounts of cineole besides small amounts of monoterpene hydrocarbons and do not show any α-terpenyl acetate. The contribution of monoterpenic hydrocarbons for the overall flavour of cardamom oil is very small. Cineole as such is a valuable material used in pharmaceuticals. A valuable and simple method of removing excess cineole in the initial stage offers attractive possibilities for commercial exploitation. An initial fractional steam distillation of the ground seeds of cardamom for 2 to 2.5 min. is recommended for improving the quality of the oil of cultivated Malabar type. Sensory evaluation studies of the fractions as well as detailed investigations of samples of cultivated Malabar type from different areas are in progress.

TABLE 1. PHYSICAL CHARACTERISTICS AND G.L.C. DATA OF DIFFERENT FRACTIONS OF CARDAMOM OIL

	Oil from Malabar type					Oil from Mysore type
	Batch 1	Batch 2		Batch 3		
	Total oil	Fr. 1	Fr 2	Fr. 1	Fr. 2	
Seed Wt. (g)	100	200		200		—
Time (min)	120	2.5	2.5-120	3.5	3.5-120	—
Oil Yield (ml)	8.5	6.5	10.5	8.0	9.5	—
n_D^{26}	1.4595	1.4575	1.4603	1.4575	1.4605	1.4605
$(\alpha)_D^{26}$	+19.10°	-1.90°	+29.10°	-1.5°	+30°	+26.4°
d_{25}^{25}	0.8941	0.8738	0.9122	0.8740	0.9177	0.9212
1,8-cineole (%)*	67.9	78.86	47.51	75.91	43.43	48.97
1,8-cineole (%) by o-cresol method of Cocking	51.1	—	36.8	—	34.4	—
α terpenyl acetate (%)	32.1	—	52.49	—	56.57	51.03

Varian Aerograph 1400 model; Carbowax 20M(15%) on celite column; Column temp. 162°C F.I.D., N_2 = 15ml/min; Injector temp. = 220°C; H_2 = 35 ml/min; Detector temp. = 230°C; Retention time: Cineole = 1'28"; α-terpenyl acetate = 5' 20"; Air = 300 ml/min.

*percentages are calculated on the basis of cineole and α-terpenyl acetate only.

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BOOK REVIEWS

Examination and Analysis of Starch and Starch Products: by J. A. Radley (ed), Applied Science Publishers, London, 1976, pp. vi+220.

This volume is the latest among the series of books brought out by J.A. Radley in the field of starch chemistry and technology over the past several decades. The volume has been brought out contemporaneously with two others, entitled *Starch Production Technology* and *Industrial Uses of Starch and Its Derivatives*, which together provide a comprehensive coverage of the entire field of chemistry, technology and analysis of starch and starch products.

Detailed descritions of specific methods for analysis of starch and starch products are available in a number of other books. But it is hard to get in a single volume a comprehensive discussion of the properties of starch, the analytical techniques developed therefrom, the theory behind them, their evolution over time and their details. It is this void that this volume fills so eminently and herein lies its great merit.

There are seven chapters in the book, each written by a specialist, a fact which adds to its value.

The first chapter deals with microscopy of starch, written by G.E. Moss. Here apart from the methods and the results for several familiar starches, a brief introduction to the theory of light microscopy is also presented. Next comes a description of electron micoscopy is also presented. Next comes a description of electron microscopy of starch by D. J. Gallant and C. Sterling (Chapter 2). Here again a fairly detailed discussion of the theory of electron microscopy (both transmission and scanning) is presented, which makes it possible even for the novice to understand the proceedings.

A. H. A. de Willigen gives a thorough presentation of the rheology of starch in Chapter 3. Study of starch is nothing without a study of its rheology, and de Willigen with the long history of important contributions to the field by the Dutch school to which he belongs, makes a lucid presentation of the entire theory and practice of starch rheology, including that of starch in the air-dry state.

Next follows a very comprehenisve presentation of the great array of physical methods of characterising starch by J. A. Radley himself (Chapter 4). It includes various methods to measure single-point viscosity, cold-paste viscocity, viscosity curves, gel properties, gelatinisation temperature, pH, colour, etc. F. A. Lyne then presents (Chapter 5) various chemical analytical methods of starches, including determination of moisture, minerals, acidity, the alkali-labile value, the alkali number, amylose, pentosans, carboxyl groups and damaged starch.

Methods for quantitative determination of starch in various products are then reviwed by F. A. Lyne in Chapter 6. The various gravimetric, polarimetric and hydrolytic methods are explained and described in detail. Finally, in Chapter 7, J. van der Bij gives a review of the various methods available for detection and analysis of the starch derivatives, viz. various starch esters and ethers.

As mentioned earlier the special value of the book lies in its approach of treating the subject of starch analysis as an organic whole. The book is not a compendium of numerous disjointed methods—good enough for the specialist and the expert who knows precisely what he is looking for and why, and who is merely interested in the details of a method—, but a coherent presentation of starch properties leading to the techniques, their theory and their description. This treatment makes it possible for even the debutant researcher to look for and decide what and how to do based on a comprehensive understanding of the subject as a whole.

All in all the book is a most valuable addition to the literature on starch.

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Proceedings of the Second International Symposium on Nitrite in Meat Products,: September 7-10, 1976 edited by B. J. Tinbergen and B. Krol., Centre for Agricultural Publishing and Documentation, Wageningen, 1977.

Reports started appearing in 1950s implicating nitroso compounds as possible carcinogens and later their presence in foods was confirmed. Since then a great deal of effort has been devoted to the fate of nitrites in foods, particularly meat. Nitrite has been shown to react in biochemical systems yielding nitroso compounds. With the present state of knowledge on these subject since nitrite is practically irreplaceable as an additive to meat the usefulness of periodic symposia on the subject cannot be over-emphasised.

The book under review records the proceedings of the Second International Symposium on Nitrite in

Meat Products organised under the auspices of Research Group for Meat Products, Zeist, Netherlands. As would be expected the Symposium had international participation and in all 36 papers were presented.

The first Session dealt with microbiological aspects and role of nitrate/nitrite in inhibiting the growth of certain pathogens.

The technological work connected with the use of nitrite has been reported in the second Session. A surprising finding of workers in USA that bacon cured with and without nitrite was rated alike goes to show that at least in some products nitrite can be dispensed with.

The maximum number of papers has been in the Chemical Session. These have been grouped under 5 sub-groups dealing with (*i*) reaction of nitrite; (*ii*) formation of nitroso amines; (*iii*) analysis of nitrosoamines; (*iv*) occurrence of nitroso amines in meat products and; (*v*) carcinogenecity of nitroso amines. The last Session reports the results of survey of intake of nitrite/nitrosoocompounds by consumers in Sweden and France. Also included is a paper on the legal aspects and the resulting experience in Norway which was the first country in the world to introduce a general ban on nitrite/nitrate in foods.

After presenting the papers in each session short conclusion and recommendations arising out of each session are given. An integrated single list of references is given at the end of the book. An editorial note is also included on the international system of units.

The printing and the get up of the book are good. The book will be of use to those connected with meat processing and those who are doing basic work on nitrites in meat.

T. R. SHARMA
D.F.R.L., MYSORE-10.

Determination of Food Carbohydrates: by D. A. T. Southgate, Applies Science Publishers Ltd., London, 1976; pp. 178.

Paucity of publications concerned with the analytical chemistry of carbohydrates contrasts sharply with the spate of publications in recent years on the analytical methodology of proteins. This book—the outcome of the author's discussions with groups interested in carbohydrate analysis during a Symposium at the National College of Food Technology, Weybridge (England) in 1972—is thus a welcome refresher.

After explaining the general plan of treatment of the subject in the introductory chapter, the author deals in a clear, critical and succinct manner with the general chemistry of carbohydrates in foods, the measurement of sugars, the measurement of starch and its degradation and modified products, measurement of unavailable carbohydrates (structural and non-structural), analysis of carbohydrates in specific groups of foods and selected procedures in seven successive chapters. Classical chemical and physico-chemical procedures as well as the modern ion-exchange and gas-chromatographic techniques have received equal attention. In the chapter on selected methods adeuqate practical details are given of procedures chosen on the basis of the experiences of the author and his close associates. Although this suffers, to some extent, from the drawback of personal bias, the chapter is bound to prove the most valuable part of the book.

The normal run of food analysts would perhaps like to see, in subsequent editions, a more expanded description of the carbohydrates in specific groups of foods and the related analytical procedures than is given in chapter 7, in a book devoted exclusively to determination of food carbohydrates, if this could be achieved without unduly swelling the size of the book which is now quite handy.

Altogether, the monograph is a valuable addition to the food analysts armamentarium and could be unhesitatingly recommended not only to the food analyst but also to the food scientist and technologist.

M. V. L. RAO

Hand Book of Food and Nutrition: by M. Swaminathan M/s. Ganesh & Co., Madras-17 Price Rs. 12 pp. 288.

The author, with his vast experience and knowledge on the basic and applied aspects of nutrition over 40 years, has summarised in this elementary text-book an integrated information on nutrition for young undergraduate students of agriculture, home-science and extension workers.

With an adequate introduction to the basic principles, the first eight chapters describe the basic facts of physiology and biochemistry of nutrients. This is followed by ten chapters (Chapters 9 to 18) devoted to nutritive value of foods, recommended dietary allowances and nutrition of specific population groups. The latter part of the book (Chapters 19 to 25) deals with special nutrition problems like obesity, geriatic nutrition and therapeutic diets. Special attention is given to diet and nutrition in India and prevention of malnutrition in developing countries. The last chapter gives information

on nutritive value of common Indian recipes and appendices give food composition tables and other relevant information.

Each chapter is brief and contains all the salient points with facts and figures. For this reason, it is an ideal book for someone who is interested in the subject but who is not an expert. As it is inevitable in a book of this nature, where it is necessary to condense a vast information in simple style understandable to the beginners, the author has obviously made a successful attempt to make each topic reasonably clear. Apart from the overall clarity, the greatest feature of this book is that it includes a number of practical hints and has provided fairly up-to-date information on current approaches to applied nutrition projects. Since this hand book will be mainly used by the Indian students and teachers, the author has rightly put the emphasis on nutritional problems in India. However, he has also described in brief the importance of correct nutrition in afluent communities by describing the problems like obseity and cardiovascular diseases.

The book can be recommended with confidence as it would not only meet the requirements of the syllabus in Nutrition of undergraduate courses, but it would also be useful for dietitians, public health and community workers and practising doctors.

P. G. TULPULE
NATIONAL INSTITUTE OF NUTRITION, HYDERABAD.

Tea and Soluble Tea Products Manufactures by N. D. Pintauro, Noyes Data Corporation New Jersey, USA—1977.

Noyes Data Corporation have been pioneers in the presentation to scientific and commercial world the US patent literature on many subjects and the present volume has put in a compact bound form the literature on patents on tea and soluble tea products manufacture.

Data have been presented on various steps in the manufacture like withering, rolling, fermentation, firing, extraction, tannin-caffeine precipitates, concentrations, dehydration, recovery of aroma, agglomeration and aromatisation and tea bags. At the end the US and UK patent indexes have been presented.

In the chapter on fermentation details of the process on improved enzyme concentration, oxidative convertion with oxygen, H_2O_2, potassium permanganate and suppressed thearubigin have been described. Of the above processes which one is commercially viable has not been indicated.

Countercurrent extractor, two steps extraction process, extraction under CO_2, solvent extraction and ammonia extraction are some of the methods mentioned. A chapter on hard water tolerance of tea products describes methods available to prevent undesirable grey lemon colour due to hardness of water used in brewing.

An important chapter of consequence to cold soluble instant tea manufactures is the problem of tannin—caffeine precipitate known as "cream". Many patents are available to solubilise the cream and also to remove the insoluble cream. Direct precipitation methods, oxidation process, alkali solubilisation, tanning treatment, polyamide complexing and polyvinyl pyrolidone processes are the significant methods mentioned. Out of these the commercially successful ones have not been mentioned.

Concentrations by hydrophilic membrane, freeze concentration and pectinase treatment have been patented.

In drying, patents on control of bulk density, vacuum belt drying, foam mat drying and freeze drying are the subjects covered.

Various techniques in improvement of soluble tea manufacture with recovery and adding back volatiles have been discussed. Whether these patents are in commercial use to give a instant tea with original flavour has not been evidenced in the commercial samples. In agglomeration techniques, General Foods Processes, using steam, salad process using vibratory techniques in humid atmosphere, spray drying chamber method are described.

Infusion tea bag construction, tea by receptacles and tea interlocks, are described with diagram.

The coverage has been up-to-date and adequate. The contents will be useful to all scientific workers employed in tea research and tea industry. The value of the matter could have been enhanced if the author had pointed out the patents in commercial use so that prospective users can make use of them. It is a useful review for any library dealing with food science and technology.

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

Industrial Enzymes Recent Advances—Chemical Technology Review No. 85 by Jeanne C. Johnson, Published by Noyes Data Corporation, Park Ridge, New Jersey, U.S.A. 1977 pp. xii+347.

"Industrial enzymes, U.S.Patent literature 1970–76" would more accurately describe the contents of this

book. It is an excellent summary of over 200 United States patents which deal with the rapidly expanding field of industrial enzymes. Journal literature, patents from other countries and U. S. patents on enzymes used for medical purposes or for clinical analyses are not covered. It is claimed that the survey is comprehensive and includes almost all U.S. patents on industrial enzymes for 1970–76.

The patents are classified into 11 sections. The first deals with general processes for recovery and isolation of enzymes and the next three with enzymes immobilised on inorganic materials, natural polymers and synthetic polymers. The remaining sections deal with different classes of enzymes, such as those acting on sugars, starches and higher polysaccharides, proteolytic enzymes for detergents and, other proteolytic enzymes, other hydrolases and other enzymes. Apart from a few introductory paragraphs, the book consists mainly of summaries in non-legal language of each patent, which include a general description of the process, examples with experimental details and Tables and Figures where necessary. Critical assessment of the patent literature is not attempted but the summary of the technical information of the patents is brief but excellent.

The production of this book in such a short time as to include processes patented in 1976 is commendable. It includes a company index, inventor index and U.S. patent number index, but no subject index other than the list of contents. The latter will only be of limited value. The classification is occasionally arbitrary (chitinases are included in 'other proteolytic enzymes' and not under enzymes acting on polysaccharides). The same subject is included in more then one section (isomerization of glucose to fructose is dealt with in 'Enzyme processes for sugars' and also on page 16 under 'General Processes for Flocculation of Enzymes'). A separate subject index would, therefore, add to the value of the book.

As indicated above, the scope of the book is very limited, but within this restricted scope, it is a very useful compilation of US Patent literature and can be recommended as essential to all those working on industrial enzymes.

V. JAGANNATHAN
NATIONAL CHEMICAL LABORATORY, POONA.

Wholesomeness of Irradiated Food: Report of a Joint FAO/IAEA/WHO Expert Committee. Technical Report Series 604, 1977 World Health Organisation, Geneva. Price $ 2.40.

This slim 44-page booklet summarises the concensus of current expert thinking on the question: how safe are irradiated foods? India is deeply interested in the matter for several reasons. For one thing, with burgeoning stocks of cereals, conventional storage methods using insecticides and fumigants are proving expensive and perhaps not too safe. For another, a leading Indian organisation, BARC, has put in a lot of time and effort into study of many aspects of food irradiation as a means of preservation of wheat, potatoes, onions, fruit and fish, and a point has been reached at which a hard decision has to be taken on whether to go in for the technique on a national scale. To complicate matters, the National Institute of Nutrition, Hyderabad, the major research organisation of the Indian Council of Medical Research, has published certain disquieting findings in regard to freshly-irradiated wheat which have given rise to unseemly controversies in the lay press. The Expert Committee included representatives both of BARC and NIN.

The evaluation on irradiated wheat in the Report runs thus: "Unconditional acceptance of wheat and ground wheat products irradiated for the purpose of disinfestation with a maximum irradition dose of 1 kucy (100 krad)". This evaluation is preceded by sections summarising data on the purpose of irradiation, the irradiation data (dose and type), microbiological aspects, nutritional matters and toxicological aspects. Thus the Report states that while the Committee had noted the increase in frequency of polyploid cells in animals eating freshly-irradiated wheat, this phenomenon had not been found by all investigators, and had disappeared when the wheat was stored for 12 weeks, a period which is likely to occur in practice. Nor was the toxicological or other significance of the increase in polyploid cells quite clear, since there is known to be a very wide range in its incidence even between normal groups of the same animal. The Report recommends that the factors involved should be investigated. Following this comes the unconditional acceptance that has been quoted. A similar format is followed for each food commodity.

There is similar unconditional acceptance of irradiated potato (15 krad), chicken (700 krad), papaya (100 krad) and strawberry (300 krad); provisional acceptance of onion (15 krad), codfish and mushrooms (220 krad) and rice (100 krad) and no recommendation for mushrooms because of limited data. Recommendations are made for future research and on what international agencies might do.

The chapters on evaluations are preceded by cogent reviews of the general and technical aspects of the irradiation technique *per se*, and of its chemical, nutritional,

microbiological and toxicological implications. Basically this is a book by and for specialists, though it may find a larger audience in India because of the importance of the issues which it debates.

K. T. ACHAYA
PROTEIN FOODS ASSOCIATION OF INDIA, BOMBAY.

Specialized Sugars for the Food Industry: by Jeanne C. Johnson, Food Technology Review No. 35, Noyes Data Corporation, Park Ridge, New Jersey, USA, 1976, pp. xii+360.

"The book serves a double purpose in that it supplies detailed information and can be used as a guide to the US Patent literature (since the late 1960s) in this field. By indicating all information that is significant and eliminating legal jargon and juristic phraseology, this book presents an advanced, commercially oriented review of specialised sugars as depicted in the US Patents" (FOREWORD). This is a fair representation of the scope of the book and this has largely been achieved.

Fourteen topics have been covered. The processes for the production of invert sugar, high and low dextrose syrups, dextrose, fructose, maltose and related oligosaccharides receive predominant coverage (about 150 of the 220 patents). Other aspects reviewed are xylose, arabinose, lactose-lactulose, cellulose, saccharification, hydrocarbon fermentation, sugar alcohols and acids.

Three methods of preparation of invert sugar (viz. acid, invertase and ion exchange resins) and the latter's use in bread mixes and fondants are dealt with in the first chapter. Separation and crystallization of dextrose and fructose from invert sugars are mentioned.

The next six chapters deal with products derived from starch hydrolysates. The use of enzymes and acids to hydrolyse starch for the preparation of low (DE $<$ 40 per cent) syrups, the isolation of therapeutic glucose polymers, cereal coatings, beverage mixes etc. are described. The procedures for the production and spray-drying of high dextrose syrups (DE $>$ 43 per cent), their use in soft drinks and stable iron-corn syrup compositions, and crystallising dextrose from syrups have been reviewed.

The enzymatic isomerisation of glucose to fructose is apparently in commercial practice for the production of high fructose (corn) syrups for use in a variety of food and beverage industries; chemical isomerization is also indicated. Possibilities of enhancing chemically the fructose content from 45 per cent (equilibrium value) to as high as 85 per cent of total sugar are indicated. Preparation of fructose powders, athletic supplements, low cariogenic sugar composition and surfactants (sucrose fatty acid esters) may be other possible outlets.

Maltose-rich low glucose starch hydrolysate syrups which are of value in soft and hard candies, frozen deserts, and in brewing and baking industries are outlined. Preparation of these syrups enzymatically, crystallisable and spray-driable and injectible maltose preparations, maltotetraose and fructose/sucrose bonded oligosaccharides have been described. These may have a good future.

The procedures for the production of lactose have been mentioned for different end uses such as infant foods, 99+per cent pure lactose, highly water-soluble preparation, an incipient, for tabletting in pharmaceutics. The preparation and use of acid-stable immobilized β-galactosidases in combination with glucose isomerase to produce a sweet product from the insipid lactose are briefly dealt with.

Xylose from sulfite liquors, cotton hulls and stone fruit shells, the use of oxalic acid for hydrolysis, purification and crystallization of xylose are difficult and expensive processes and have been detailed.

Maple syrup, tablets or biscuits from honey, sorbose ketals, use of fungal pellets as β-galactosidase sources to hydrolyse raffinose in sugar beet syrups, gentiobiose, D-arabinose and D-ribose (for riboflavin production), sorbosone (for ascorbic acid), D-mannose, maltol (sweetness enhancer) are other products described.

A few exotic futuristic processes are indicated. Examples are glucose production using immobilised "cellulase" or mixed cultures. aerobic fermentation of hydrocarbons to sugars, amino acids and sugar esters, edible polysugar condensates, photochemical (UV) formation of carbohydrates from CO_2, preparation of bifidogenic lactulose and the non-cariogenic non-insulin requiring sweetener xylitol, non-absorbed sweetener maltitol, sugar alcohols such as mannitol (a bodying and texturing agent), sugar alcohol esters, gluconic acid production by immobilised glucose oxidase—catalase, therapeutic pangamic acid, gluconate containing spray-driable starch hydrolysates, 2-keto gluconate from sorbosone, 2,5-diketogluconate, salicylate-mannuronates of alkali metal methylsilanolates as potentiators of other pharmaceuticals. Some of these are intensively pursued and may have good prospects.

Inclusion of the following would have made the book more useful. Processes which are under commercial exploitation and seem to have immediate application should have been indicated: perhaps invert sugar, glucose and related products, high fructose syrups and maltose derivatives are commercially made now. Indications of comparative costs for different processes for a product

would have been a welcome and useful feature. There is (was ?) a controversy in the European Economic Community regarding high fructose corn syrup *vis-a-vis* beet sugar. Chemically modified starches, gums and polysaccharides and their uses in food industry do not figure here at all. Coverage of enzymatic and microbial cellulose hydrolysis is meagre despite voluminous literature: perhaps these have not been patented. A subject index would have been useful despite the inclusion of a detailed table of contents and other indexes. One wishes that a few (detected!) wrong statements were not there: e.g., page 3, para 2, line 6—"*hydrolysis* of glucose" in place of "isomerisation"; page 90, para 1, line 3—"in the free state, it (dextrose) occurs in honey, fruits, *starch* and *cellulose*"; page 130, line 4—"1973 to 14.5 *cwt* in 1975" in place of 14.5 *million cwt*; page 350, para 4 and line 2—"Dowex-50 is a *polyacrylic* resin..... "instead of "*polystyrene* resin.....".

These comments do not detract from the usefulness and the wide scope of the book. The book contains much useful scientific and technical information on a variety of sugars and related processes culled from the US Patent literature. The high cost notwithstanding, it is commended to all interested in carbohydrates.

M. R. RAGHAVENDRA RAO
C.F.T.R.I., MYSORE.

Package Production Management: by Harold J. Raphel and David L. Olsson, AVI Publishing Co. Inc., Westport, Connecticut, 2nd Edition, 1976, pp. 250.

This book, formerly entitled "Packaging—A Scientific Marketing Tool", is an up-dated version of the edition. It is a welcome edition to the literature on packaging. While updating the original edition, the authors have taken into account the enactment of new laws which affect packaging and regulations imposed by environmental/ecological concerns (solid waste, disposal, litter, use of resources, consumer safety).

The objectives of the authors are to present information concerned with the proper use of packaging as an aid in the marketing of consumer products, functions of the package and package development, management of package function, package design and selling, package research and testing, package graphics and reproduction and the need for packaging professionals. These objectives are achieved to an admirable extent by tying together the information in concise and lucid manner with some illustrations in the ten chapters of the book.

Each chapter begins with a short introduction and ends with a bibliography except chapter 10.

The first chapter entitled "Packaging and Our Changing Nation" discusses the effect of consumer trends on packaging of goods. This Chapter also describes how the future consumers will influence the packaging needs and how this can be achieved by advancing technology of packaging.

The second chapter entitled "Development of the Package as a Sales Tool" deals with the historical development of the package to its present state, packaging as an economic force and as a service, how packaging can help implement "the four P's of marketing".

In the third chapter entitled "How Shopping Decisions are Made", the authors summarise some of the prevalent view points and how package can influence the consumer behaviour.

The fourth chapter deals with "Package Development". The authors describe the functions of a package and restrictions and regulations of package and packaging systems.

The fifth chapter "Management of the Packaging Function" deals with long range packaging planning, the package development programme and management of packaging functions, the role of top management in packaging and some recent trends in packaging organisations.

The sixth chapter entitled "Areas of Special Importance to Packaging" deals with those topics which are of special importance to any packaging and marketing programme.

The seventh chapter entitled "Package Design for Selling" deals with the importance of package design which attracts the prospective buyers. This chapter also deals with two major subjects like essentials of good design and surface designs of a package.

Chapter eight of this book deals with the subject "Package Research and Testing" and the requirements of such testing during packagers', work in the areas of protection, production and quality control. A few photographs of equipment used for testing packages are also given. The chapter ends with discussion on research and testing, marketability of the package.

Chapter nine deals with the subject of "Package Graphics Reproduction" which includes printing processes, preparation of copy and printing plates, package printing and printing quality.

The last chapter (chapter ten) is a very short one consisting of four pages. The authors discuss the need for packaging professionals in this chapter.

The printing and get up of the book are very good anp will be a valuable asset for students specialising in pack-

aging, people concerned with marketing and packaging professionals.

S. C. CHAKRAVORTY

Food Flavoring Processes: by Nicholas D. Pintauro, Noyes Data Corporation, New Jersey, 1976, pp. 209,

The book under review No. 32, in the Food Technology Review Series from these publishers, is a survey of United States Patents on Food Flavours, from 1961 to 1975 and presents the essence of 214 selected patents, omitting the patent jargon. The information is essentially on the (*i*) synthetic compounds in the different formulations and examples of application of the formulations and (*ii*) the formulation of specific aromas through use of enzymes and fermentation and heating of specific combinations of raw materials and chemicals. For ease of reference the information is classified (with subclasses) under aroma types as, fruit and vegetable, dairy, bread, meat, nutty, fried and roasted, coffee, chocolate and tea flavours. Formulations for seasoning, flavour enhancement, freshening and masking requirements and flavour emulsion and fixation are also covered.

In view of diverse flavor applications of many compounds through small variations in structure or concentrations, cross reference to patents in other chapters with possible applications to the flavour discussed in a particular chapter is given in the beginning of each chapter.

The book is a good source of reference for research and development of flavoring compositions. It would be more helpful if the GRAS status of the substances mentioned in the chosen patents are given and the budding flavorist is cautioned on the many pitfalls in the application of the flavor substitutes through a bibliography of suitable references. A chemical index would add to the easy use of the book.

V. S. GOVINDARAJAN
C.F.T.R.I., MYSORE.

ASSOCIATION NEWS

Hyderabad Chapter

We welcome the News Letter brought out by the Hyderabad Chapter in mimeographed form for the benefit of the members. Besides covering news items about the Chapter, it also includes scientific and technical aspects of food and anwers to the queries forwarded by the members.

Souther Branch

Talk delivered by Shri K. L. Radhakrishnan, General Manager, Modern Bakeries (India) Ltd., Madras on Fortification of Foods on 23rd Dec. 1976.

Undernutrition and malnutrition are among the important problems facing many developing countries like India. The availability of foods has not been able to keep pace with the ever increasing population. Poverty is wide spread and people are caught in a viscious cycle of "Poverty-malnutrition- diseases". About 80-90per cent of the average Indian diet consists of cereals with very little of protien rich and protective foods. Nutritional disorders like kwashiorkor, night blindness, anaemia, etc., are quite common among vulnerable sections of the population whose growth and health are stunted and crippled. It is estimated nearly 10,000 people die everyday in the world due to malnutrition.

The solution to the problem of undernutrition and malnutrition lies not only in increasing the availability of foods by increased Agricultural Production but also protecting their nutritional quality by adopting scientific methods of processing and preserving foods. Losses of nutrients taking place during processing of foods can be made up later on by enriching them. The nutritive value of foods can also be improved considerably by fortifying them. Enrichment and fortification of foods can thus play an important role in overcoming the problems of malnutrition.

Fortification denotes tne addition to a food of one or more dietary essentials in amounts which make the total content greater than that found in that particular food in its natural state. It can also mean the addition of one or more dietary essentials to a food which the latter does not contain in its natural state. The term "enrichment" on the other hand, is used to signify the addition of dietary essentials to a food to restore the total content of the former to the levels obtaining in the food in its natural (unprocessed) state.

Seven foods can be thought of for enrichments and fortification and some of the attempts made in different countries in this regard are discussed.

Enrichment of rice with thiamine, niacin and iron was developed and used in the Philippines in 1947–50 to cure the wide spread disorder, namely, Beri-Beri. Wheat *Atta*, *Maida* and Bread can be fortified to provide better nutrition. During the world war II, the United Kingdom laid down that all flour shall be of 85 per cent extraction leaving enough of thiamine, white flour fortification with thiamine and calcium was resorted to on a national scale. In U.S.A., all refined flour is fortified with vitamins B_1 and B_2, niacin, iron and calcium. In India, *Atta* ground for Defence Services has been fortified with calcium carbonate since 1946. In 1970, the Government of India arranged for the production of fortified *atta* with various vitamins and mineral salts and also edible groundnut flour to improve the protein content. Modern bread is a standing example of a fortified bread where protein, vitamins A, B_1 and B_2, niacin and iron are added in large scale production.

Examples of other foods that have been fortified are: Vanaspati with vitamin A and D skim milk powder with vitamin A and salt with iodine or calcium or iron. Apart from these, several processed foods like infant and weaning foods, break-fast cereal foods, etc., put out on the market are all fortified with various nutrients. Fruit juices, squashes and carbonated beverages have also been fortified by enterprising manufacturers in developed countries.

Factors which need attention for successful implementation of any fortification programme are:

(*i*) A centralised agency to organise production and distribution of fortified foods;

(*ii*) fortified foods should reach maximum number of consumers;

(*iii*) nutrients added for fortification should be stable during storage, processing and cooking of the food;

(*iv*) fortified foods should not be different in appearance, colour, taste, etc to the foods people are used to; and

(*v*) fortification should not be very expensive.

Fortification of foods can thus serve as an easy method in correcting the nutritional deficiencies of the average diet and thereby improving the health of the masses. It has an important role in mass feeding programmes like Midday meal programmes in schools, special nutrition programme for the benefit of preschool children and expectant and nursing mothers, etc., being implemented in our country.

Talk given by Shri S. T. Chari, Joint Director of Fisheries on Recent Trends in Fish Processing Technology on 19th Feb. 1977.

The speaker prefaced his talk with the background information on the vast fisheries resources of this country which at present stood at 2.20 million tonnes per annum comprising of 1.6 million tonnes of marine fisheries and the rest being from the Inland and brackish water sources. Fish is a highly nutritious item of food, but a highly perishable commodity requiring immediate attention if the quality is to be preserved till it reaches the consumer. The merits and demerits of traditional methods of preservation like plain drying, salt curing and drying were traced and the speaker highlighted the recent trends and the developments in fish processing Technology.

Freezing of fish, especially prawns, has been readily adopted by the fish processors in this country since prawns, which is a highly valued delicacy in the Western countries, fetch a high unit value, if this could be sent in as fresh condition as possible. Freezing as a method of preservation is being adopted and by many processing establishments in Kerala and in the East Coast. Beginning from a scratch of export value of Rs. 50.00 lakhs in 1956, India exported in 1974–75 about Rs. 120.00 crores worth of prawns and this has been possible due to the recent advancements in the design of the freezing equipments, construction of cold storages, transport facilities and reefer space in ships.

The improvements in plate freezing of prawns in the shortest possible time through its critical freezing zone of –1°C to –5°C was stressed to avoid formation of big ice crystals in the muscle and thus prevent nutritional and quality loss on thawing. The frozen material is kept in storage at –30°C, at which temperature the denaturation of proteins and loss of such characteristics as texture, flavour and dehydration are kept to the absolute minimum. In this, liquid nitrogen has been found successful as a refrigerant.

Canning of fish has advanced to a very great extent in all developed countries and in India though quite a large number of factories have been established for canning fish, this has not become quite popular for internal market as well as for the export trade, for the simple reason that the cost of the tin container and the medium used like oil, tomato sauce etc., is disproportionate to the cost of fish inside. But the technology of caning of fish to produce highly sterlized product that can be preserved for 2 years has widely been acknowledged. Fish canning has got special problems like lacquering inside to suit different types of fishes like prawns, crabs, meat, tuna, cat fish etc., and processing temperature, water-oil ratio etc., which have been sucessfully overcome.

The speaker mentioned about the latest technique of preparation of fish sausage which is widely practised in Japan, for which fish flesh from all types of fishes devoid of bones, skin, etc., is minced, mixed with a starchy medium, salt, spices, fat and a chemical preservative like AF_2 (permitted in Japan) and heat treated after filling in 'saran' or synthetic or animal casings. The material is flash sterlised and stored. Shelflife of the product at room temperature is about 3 to 4 weeks. An attempt has been made in this country to produce this at the Fisheries College at Mangalore. The problem faced is the availability of indigenous casing material and also use of the chemical (AF_2) which has not been cleared by the Government of India.

The preparation of edible fish flavour or fish protein concentrate from all varieties of fishes by several extraction methods using solvents such as isopropyl alcohol, or alcohol and deodorising it by steam stripping and vacuum evaporation was described. It will be possible to use miscellaneous varieties of fishes which would at present be considered to be unimportant and uneconomical. It is important as a valuable source of animal protein to bridge the protein gap. Experiments in human food trials at Kerala Medical College, Trivandrum have been found to be successful. Fish protein concentrate is a vaulable additive in such preparations as bread, biscuits, soups, flakes, fish cakes, etc.

In order to utilise certain marine fishery resources during heavy landings, plants for the industrial production of fish meal for feed purposes of cattle, poultry etc., have been established. Tamilnadu has the biggest Fish Meal Plant in India, at Mandapam where a variety of fish called silver bellies and other miscellaneous varieties are being processed into fish meal. This is a modern plant that could produce fish meal of good quality besides yielding fish oil as a by-product.

Quite recently as a result of the technological research carried out by the State laboratory, the preparation of a silage product from fish has been standardised. Fish is minced as such, molasses and water are added and boiled. A culture of *Lactobacillus plantarum* is added after it attains to ferment for about 72 hours when a product with a characteristic malty fermented odour is obtained. This can be kept for a period of more than 12 months. This ensiled product can be mixed with other feed rations and used as a feed for cattle and poultry.

The speaker also explained the latest developments in the artificial drying of fish in which the fish can be kept in trays in tunnels where flow of conditioned air at a certain temperature, relative humidity and velocity

is maintained and product has been found to be uniformly dried and hygenically prepared, free from infestation and of the desired dryage and appearance.

There was a lively discussion on the utility of idle-capacity of cold storage and canning factory for other purposes like storage of mango pulp and canning of fruits, vegetables, etc. at the freezing plants established for the fish preservation purposes. It was mentioned that since fish and other items of food materials do not go hand in hands due to odour problem the fish processing plants are utilised at present for fisheries purposes only.

On the question of flavour reversion in fish protein concentrate, the Speaker explained that this problem occurs sooner or later depending upon the solvent, used, stream stripping and vacuum drying techniques adopted, etc. The use of certain anti-oxidants, packing in an inert medium, proper storage conditions, has mitigated this problem to some extent. However prople in Africa and the far east do not mind such a flavour. On the question of economics of the product. it was mentioned that, fish protein concentrate is costlier than other vegetable proteins, but it has got an edge over them in view of the high protein content having all the essential amino-acids profile.

Half a Century of Dairy Development in India

Shri R. Gopalan, Joint Commissioner (Retired) Dairy Development Corporation, delivered a lecture on 29 January 1977 on half a century of dairy development in India.

Ordinary Members

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Honours and Awards

Shri A. M. Nanjunda Swamy, Shri G. Radhakrishnaiah Shetty and Smt S. Saroja, all from the Central Food Technological Research Institute, Mysore have been awarded the Yezdi Award for their paper entitled 'Studies on the development of newer products from mango', published in Indian Food Packer in 1976. The Award, instituted by Yezdi Industries, Mysore, is given to the best research paper with immediate application to industry published in Indian Food Packer.

Dr. M. Mahadeviah of the Central Food Technological Research Institute, Mysore has been awarded the K. U. Patel Memorial Award by the All India Food Preservers' Association, for his paper entitled "Internal corrosion of tinplate containers with food products", published in Indian Food Packer in 1976. This award is given to the best research/review paper in food technology published in Indian Food Packer and is useful to the Food preservation Industry.

The Indian Council of Agricultural Research (ICAR), has awarded the Rafi Ahmed Kidwai Memorial Prizes for the biennium 1974-75, in the subject of Food Technology to, Shri E. S. Nambudiri, Shri N. Krishnamurthy, Dr. A. G. Mathew, Dr. Y. S. Lewis and Shri C. P. Natarajan, all working in the Central Food Technological Research Institute, Mysore, for the outstanding contribution in the field.

Second Announcement of the

Fifth International Congress of Food Science & Technology

The "Second Circular" of the Fifth International Congress of Food Science & Technology, which will be held from September 17 to 22, 1978 in Kyoto, is now available.

All who have already sent the pre-registration cards to the Congress Secretariat will receive the "Second Circular" before long. Those who have not yet finished pre-registration but will participate in the Congress should contact the Congress Secretariat as soon as possible.

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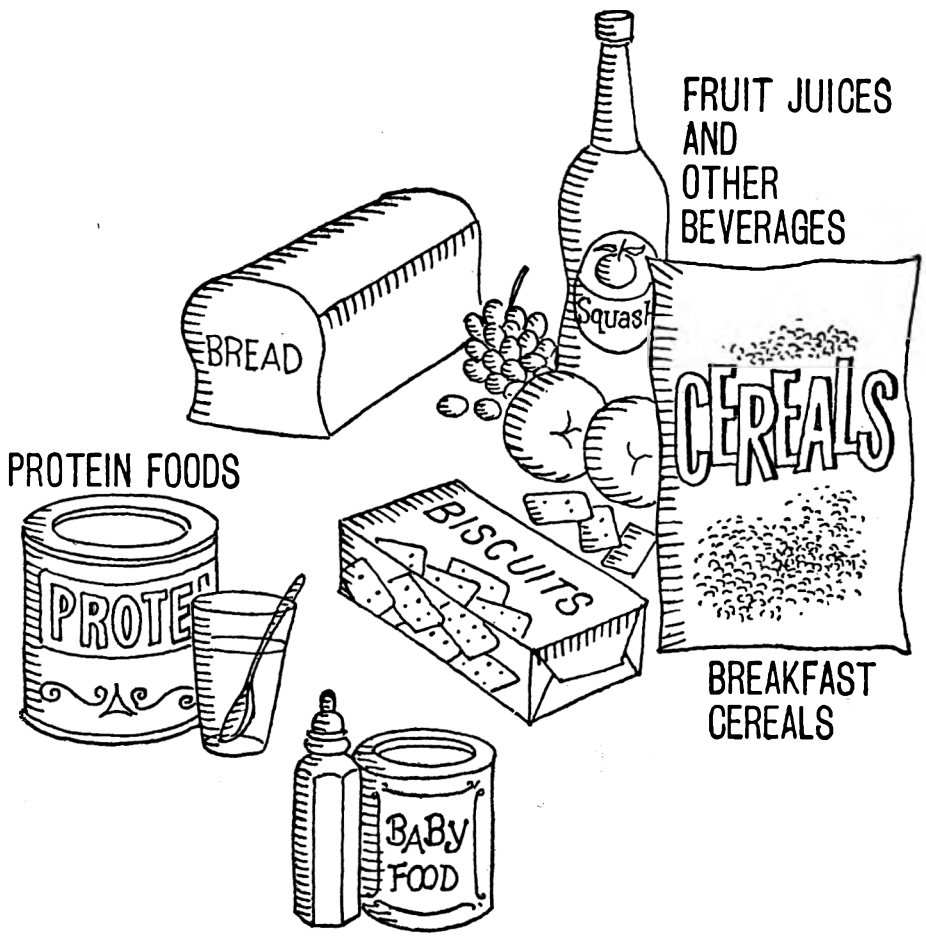

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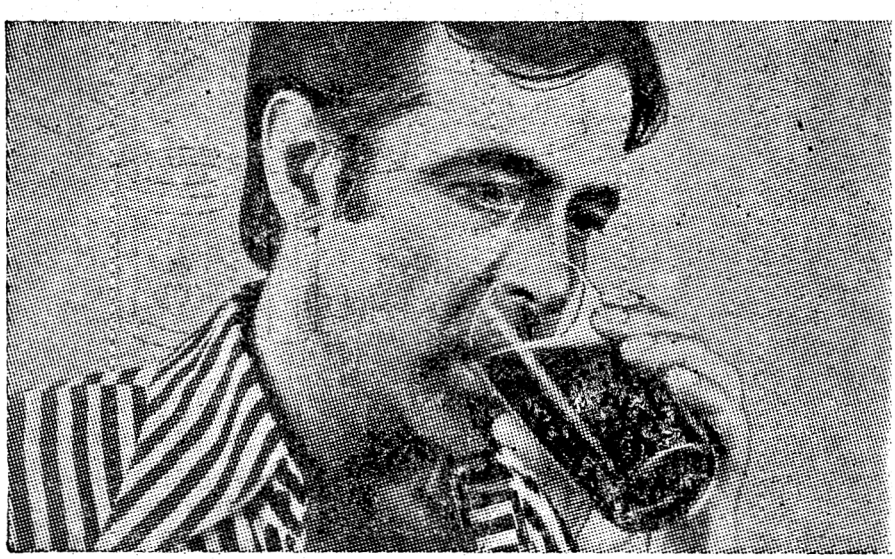

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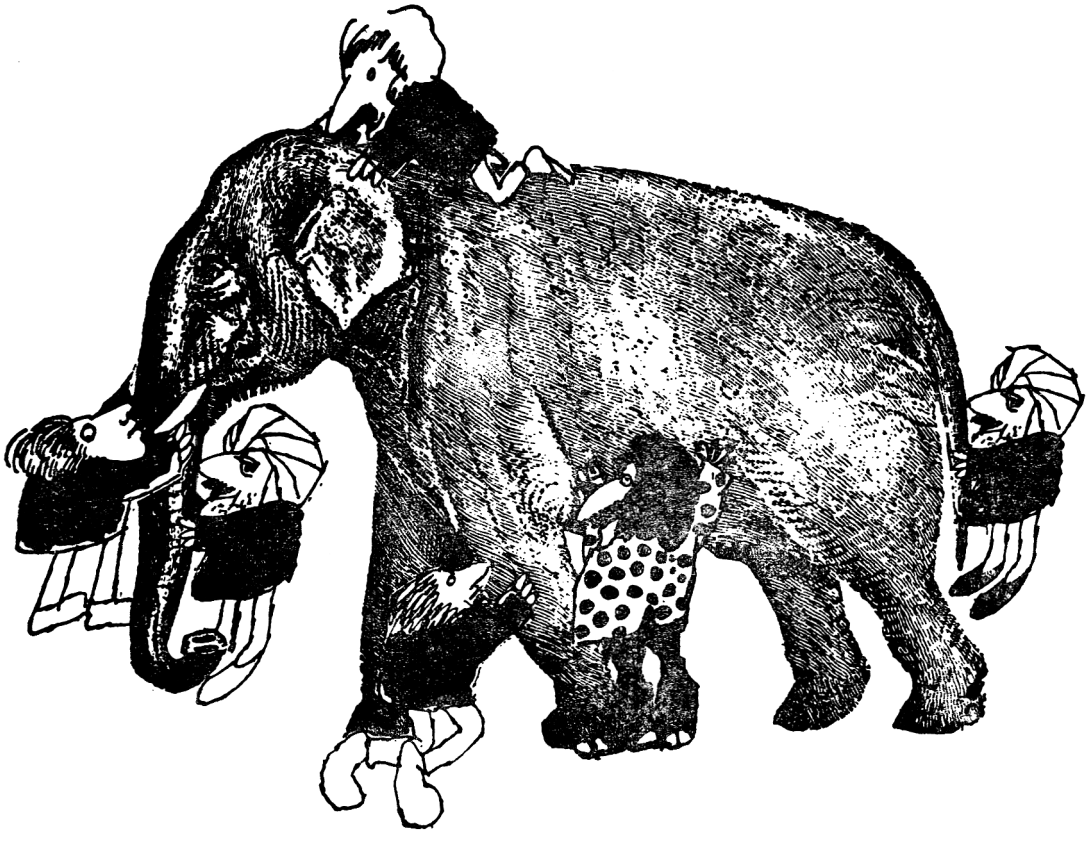

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2. Short communications in the nature of Research Notes should clearly indicate the scope of the investigation and the salient features of the results.

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.

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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.

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   (*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.

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