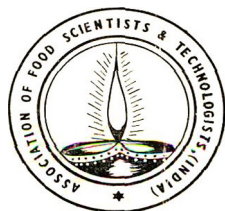


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# JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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# In-pack Processing of Stuffed *Parotta* in Indigenous Flexible Packaging Materials. Part I. Investigations on the Effect of Processing on the Packaging Materials

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*Manuscript Received: 14 September 1973*

**Systematic investigation was carried out on the effect of autoclave processing on paper/foil polythene pouches after the packs were stored in ambient condition for 6 months. It was observed that an appreciable proportion of the pouches showed leakage either at the sealed areas or across the body of the pouches. These could be attributed to thermosoftening of the polythene layer at the processing temperature (121°C). The observed spoilage could also be attributed to this effect.**

Work carried out initially in this laboratory on heat processing of stuffed *parotta* in indigenous flexible pouches<sup>1</sup> was suitable for laboratory scale experiments only. Later on some modifications were introduced<sup>2</sup>, for commercialisation. The apparent success of this work using quite an unconventional foil laminate for heat processing, demanded further critical examination of the method, including large scale practical trials. The present work was undertaken to identify and overcome the shortcomings, if any, when used on a large scale and secondly to introduce possible improvements including alternative packaging materials of indigenous sources.

Accordingly about 50,000 packs were made in several batches by the modified method<sup>2</sup> and most of these packs were sent to various user units by rail/road transport. On examination of these packs after 6 months, it was reported that 25 per cent of them were spoiled by fungal attack. The packs which were retained for static storage were therefore, thoroughly examined. The present paper describes the findings of these investigations.

## Materials and Methods

**Packaging, processing and storage:** Stuffed *parottas* made as previously described,<sup>1,2</sup> were packaged in groups of two, first in MST cellophane pouch and then in an outer pouch of 60-g kraft paper/.04 mm foil/polythene 150 gauge laminate. The processing details were exactly those described earlier<sup>2</sup>. Batches of 400 packs (800 *parottas*) at a time were processed

and random sampling from each batch followed by microbiological testing showed that they were rendered sterile. These packs were repacked in cardboard cartons each containing five number and after properly enclosing in wooden cases were sent to user units by rail/road transport but approximately 200 numbers of the cartons were retained and stored under ambient conditions (25-30°C) for 6 months. During processing, packaging and storage these packs were subjected to normal handling hazards in shifting from place to place and no extra care was taken.

At the end of 6 months storage period, 50 packs were taken out at random and subjected to the following tests.

**Leakage test under vacuum:** The packs were kept by turn in desiccators which were evacuated and the vacuum maintained for about 5 min. Initially the pouches got inflated. In case there was leakage, this was indicated by gradual collapse of the inflated pouch.

**Seam gap test:** The heat sealed seams were tested for any possible gap. This was done by cutting open the pouch into two halves; taking out the cellophane wrapped *parottas* and pouring coloured acetone into each half. Leakage of acetone at any point in the seal area indicated a gap there.

**Test for cracks and holes in the body of the laminated pouch:** This was done as in seam gap test with coloured acetone. By carefully taking the acetone

solution all along the inner surface of the two halves any hole or crack through which the acetone leaked out was detected.

*Visible pin holes in the foil layer:* This was observed by placing the foil laminate over an aperture in a closed chamber containing strong light and looking for any pinholes in a dark surrounding.

*Examination of the parotta:* General condition of *parottas*, viz., whether they were in good condition without development of any bad odour or whether they had become mouldy or were rotten with bad odour, was observed.

In addition to the above tests the polythene layers of some of the pouches subjected to autoclave processing, were separated and examined under microscope. Particular attention was directed at the white patches formed during the processing and the thickness variation at these points was observed by actual measurements with micrometer gauge. The separation of the polythene layer was carried out by thoroughly soaking the pouches in water, pulling and rubbing off the paper layer gently and then dissolving the aluminium foil with 10 per cent caustic soda solution slowly at room temperature.

## Results and Discussion

This investigation was carried out to have quantitative idea of the damages and their nature which the pouches might suffer from, in large scale operation. Kannur *et al.*<sup>2</sup> had observed that occasional spoilage was observed in some pouches due to pinholes in foil and due to improper handling of the pouches during transportation. Since in large scale operation even with normal care some amount of handling abuse could not altogether be avoided,

it was considered necessary to have a quantitative study of this aspect so that necessary improvements might be introduced to counter this effect.

Table 1 summarises the results of various tests and enables one to know the number of packs which show a particular defect, and to what extent the observed defects are interrelated.

The simple and nondestructive test employed to detect leakage in the pouches could be carried out as the packs contained some amount of air inside. However, the pressure developed inside being of a low order and also the period of observation being only 5 min, it is possible that the observed cases of leakages are only due to well defined gaps in the seal area or holes across the pouch body and do not include those having very minute holes or gaps. Surprisingly, a large number of pouches showed holes across the pouch body and a still larger number showed minute visible pinholes in the foil, though the thickness of the latter (.04 mm) in the laminate precluded this possibility. The cause of this was traced to the use of machine glazed kraft paper in the construction of the laminate used. This paper, it was learnt, due to its rough surface gave rise to a gritty finish in the final laminate and as a result, the pouches suffered damage during packaging operations. It is also obvious that some of the prominent holes observed across the laminate occurred during the handling operations.

It is interesting to note from Table 1 that though 11 cases showed faulty seam and 15 cases showed holes in the pouch body, only a small fraction of them led to spoilage of the *parotta*. Apparently this could be due to the protection afforded by the inner MST cellophane pouch. On the other hand, of the 5 cases of spoilage by mould growth, 3 could be attributed

TABLE 1. EXAMINATION OF FOIL LAMINATED POUCHES AFTER STATIC STORAGE OF STUFFED PAROTTA PACKS FOR SIX MONTHS

Test	Positive to test*	No. of pouches out of these in column 2 which show additionally a positive test in respect of				
		Leakage under vacuum	Seam gap	Holes/cracks in pouch	Visible foil pinholes	Visible spoilage of <i>parotta</i>
Leakage under vacuum	8	8	7	3	2	4
Seam gap	11	7	11	2	5	3
Holes/cracks in the pouch body	15	3	2	15	15	2
Visible spoilage	6	4	3	2	5	5 mouldy, 1 bacterial spoilage

60% of the pouches showed visible pin holes in the foil layer

\* No. of pouches out of 50, showing a positive indication in the test.

to faulty seam and 2 to holes across the pouch body. One case of bacterial spoilage could not be attributed to package failure as none of the defects looked for, was found in the pouch.

The inner polythene layer of the autoclaved pouches

was found to have developed some white patches (Fig. 1). This was observed earlier also<sup>2</sup>. The thickness at these patches measured .0018 to .0016 in. Under microscope, these patches looked like clusters of bubbles (Fig. 2). One could imagine these having

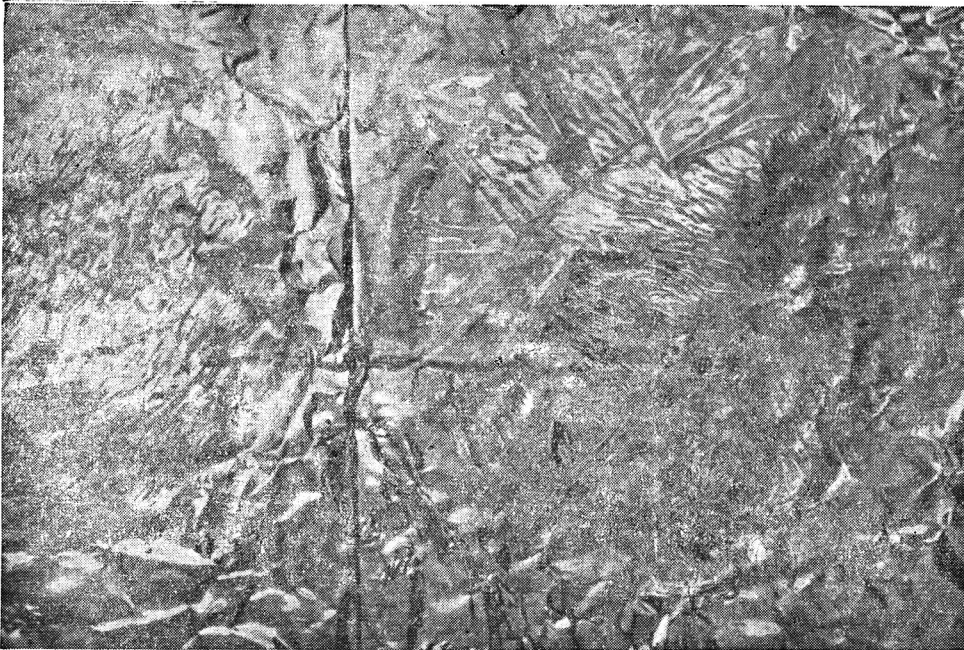


FIG. 1. Inner surface of an autoclaved pouch

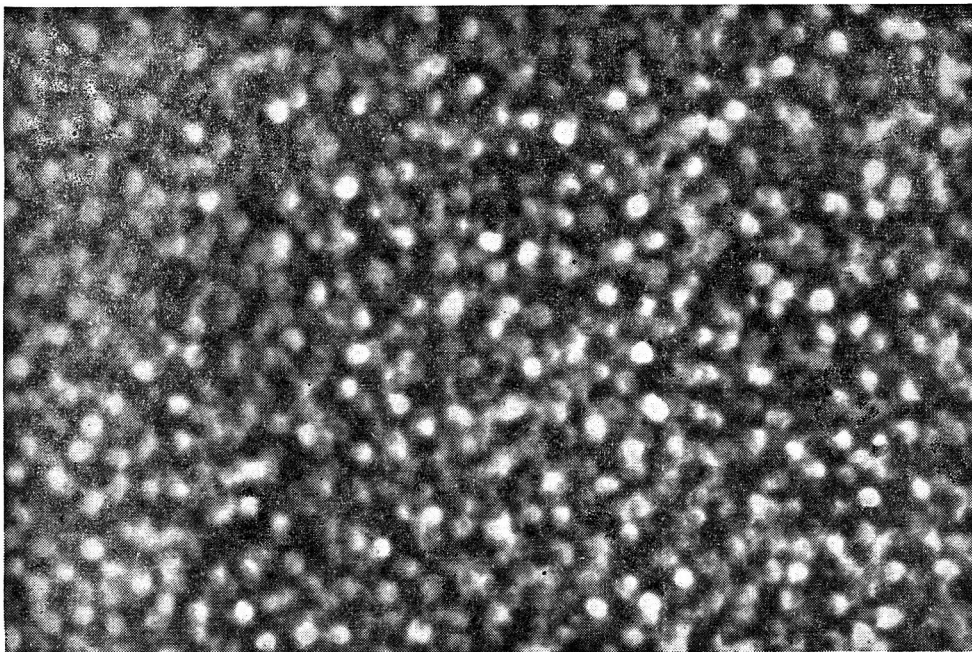


FIG. 2. Photomicrograph of white patch on the inner surface of an autoclaved pouch ( $\times 409$ )

been formed during thermosoftening of the polythene layer at the processing temperature by the action of entrapped air/gas under pressure. Formation of these bubbles accounts for slight increase in thickness at their sites. However, bubble formation could take place by reduction in wall thickness and increase in effective surface area. As an indirect evidence of this fact water vapour transmission rates were determined on the polythene film separated from the laminate both before and after heat processing. The figures obtained (in units of g/sq. m/24 hr at 37°C and 90 per cent RH) are 10.1 in the former and 57.1 to 186 in the latter case, where specimens were taken for tests especially from areas containing white patches. Thus considerable attenuation of the polythene layer at the affected places is indicated with consequent reduction in its protective power against ingress of microorganisms in case of failure of the foil layer. Proctor and Nickerson<sup>3</sup> have reported that in gauges of 0.5 (.0005 in) or less plastic films can be penetrated by microorganisms. Table 1 also shows that due to the basic weakness of the foil laminate on largescale operations there could be spoilage in 10-12 per cent of the packs due to microbiological attack within a period of 6 months even on static storage. If the packs are subjected to transportation hazards, there could be further physical damage in the packs and the resulting microbiological spoilage has been reported to be as high as 25 per cent by the users.

It is concluded that stuffed *parotta* packs made by the method previously described is susceptible to spoilage under handling operations normal in large scale production. The spoilage occurring during storage is attributable primarily to thermosoftening of the polythene layer during autoclave processing

at 121°C which leads to considerable thinning at places, or hole formation under stress or opening up of the seam at certain places under stress. The spoilage could also be attributed to faulty construction giving the appearance of minute gritty particles embedded in the laminate but this defect is remediable and therefore not of much importance. In fact in subsequent large scale operation where laminates of correct construction have been used there was no grittiness and yet spoilage of *parottas*, though to somewhat lesser extent, was observed during storage. Moreover, the integrity of pouch for autoclaving depends much upon its innermost polymer layer. The aluminium foil layer, next to it acts mainly as a barrier against oxygen, light and moisture. Besides being liable to pinholing during handling, it is not free from pin holes originally in the gauges it is normally used. In the sophisticated laminate which has been reported to have been successfully used<sup>4</sup>, the polymer layer has a thickness of 0.075 mm while the aluminium foil is only 0.009 mm thick. In foil laminate used, (0.04 mm) it is difficult to maintain the foil free from pinholes during large scale operations. Hence the innermost polymer layer has got to play a much more protective function and further work in this direction is necessary.

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# Canning Studies on Sweet Potatoes\*

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The overall means for firmness of processed sweet potatoes as measured by the ASCO firmness meter and the shear press decreased as time of storage of the roots prior to processing increased. Calcium treatment at higher levels increased the firmness of the sweet potatoes stored 0, 30 or 90 days prior to canning. Higher average pectin levels added to the canning syrup increased the shear values, and a continuous positive response was observed in ASCO firmness values for higher levels of pectin. It was shown that pectin had a significant influence on ASCO values, but not on shear values, suggesting that the parameters measured by shear press and ASCO firmness meter were different. Lower ASCO firmness values and higher shear values were obtained for a 6 min soak time in calcium hydroxide solution indicating that 6 min of soaking in the calcium solution was sufficient to firm the sweet potatoes.

Addition of zero and 3 per cent pectin to the canning syrup resulted in a slight darkening of the canned product, but Hunter 'L', 'a', and 'b' values for roots canned with pectin in the syrup were all within the commercial range.

The problem most frequently mentioned by both consumers and processors of sweet potatoes involves loss of wholeness and firmness in the product<sup>1</sup>. Product breakdown in sweet potatoes is not a new problem. Earlier work in this area was reported by Culpepper and Magoon<sup>2</sup>. Ensuring the retention of the original shape and form in processed roots is believed to be of value for better consumer acceptance.

Kattan and Littrell<sup>3</sup> showed that the length of time and temperature of storage before canning markedly decreased the firmness of canned roots. McConnell<sup>1</sup> showed that the changes responsible for breakdown and softening in processed sweet potatoes actually occurred, to a large extent, before the roots had been processed. Ahmed<sup>4</sup> showed that the breakdown of canned sweet potatoes was related to the kinds and amounts of pectic substances contained. He also reported that the length of the storage period of the raw roots prior to canning was important, because 40 per cent of the pectic substances disappeared during storage.

The application of calcium salt, in concentrations less than the U.S. recommended maximum, satisfactorily minimized storage effects and increased the firmness of canned potatoes<sup>5</sup>. La Belle<sup>6</sup> showed that the firmness of red tart cherries was increased by 50 per cent following treatment with a 0.5 per cent calcium chloride solution.

The purpose of this study was to evaluate the effects of calcium, pectin, storage time prior to processing, and the time of soaking the roots in the calcium solution on the firmness of canned sweet potatoes.

## Materials and Methods

*Raw material:* Eight bushels of the Centennial cultivar of sweet potatoes grown in the vicinity of Starkville, Mississippi, were brought to the processing laboratory on the day they were harvested. The roots were separated randomly into three lots and canned. The first lot was processed immediately and the second and third lots were stored at 21-26.6°C for 30 and 90 days, respectively prior to canning.

*Pretreatment and processing:* The three different pectin concentrations used were 0, 1.5 and 3 per cent by weight in a 25 per cent by weight sugar syrup. Concentrations of 0, 2 and 4 per cent calcium hydroxide by weight in water with soak times of 3 and 6 min were evaluated. There were 11 treatments in the experiment and for each treatment 6 number 303×406 cans were packed.

The roots were washed and peeled in 10 per cent boiling sodium hydroxide (lye) solution for 5 min. The peeled roots were washed under a cold water spray, and trimmed to remove damaged areas.

After soaking in the calcium hydroxide solution, the roots were packed into the cans and covered with

\* Publication number 2666 of the Mississippi Agricultural and Forestry (MAFES) Journal.

the appropriate hot sugar-pectin syrup. The cans were steam-flow closed and processed in a still retort for 34 min at 115.6°C. The second and third packs were processed at 115.6°C for 60 min. These processes were recommended by the National Canner's Association for freshly dug and cured roots respectively. Except for the heat process, the second and third packs were treated and packed similar to the first pack.

Five cans, containing roots from the third lot treated without calcium and pectin, were packed and processed for 2 min at 132.2°C in the Food Machinery Corporation's Steritort (Model 600-10) to evaluate high temperature short time processing on sweet potatoes. It is not known what this process yielded as no heat penetration study was made.

*Analytical:* The canned samples were stored for 75 days between 21-26.6°C after which objective evaluations were carried out. Roots from three cans per treatment were analyzed for firmness by use of the ASCO firmness meter. This instrument was standardized using a pre-stress load of 200 and a linear test load of 400 for 5 sec. Roots having approximately equal diameter, were used to obtain ASCO firmness values and root diameter was measured and recorded.

The shearpress used in this experiment was a Food Technology Corporation's Model TP-1, texture press with a standard shear compression cell. The values for resistance to shear were obtained using a 40 g sample of  $\frac{1}{4}$  in cubes placed in the shear cell with the result expressed as the pounds of force required to shear the sample.

*Statistical design:* A completely randomized  $3 \times 3 \times 3 \times 2$  factorial design was used involving 3 storage times for the roots prior to canning, 3 pectin concentrations, 3 calcium hydroxide concentrations and 2 soak times in the calcium solution. Data were subjected to analysis of variance using the Mississippi State University Univac Computer Model 1106. Duncan's new multiple range test was used to separate the means where significant interactions were found.

## Results and Discussion

*ASCO firmness meter results:* The treatment means for ASCO firmness for storage times, calcium hydroxide concentrations and soak times are shown in Table 1. Analysis of variance of the data indicated that the interactions for storage time *vs* calcium concentration, storage time *vs* soak time, and storage time *vs* calcium concentration *vs* soak time were all highly significant. Conclusions about the single effects for storage time, soak time and calcium con-

TABLE 1. THE EFFECTS OF LENGTH OF STORAGE OF ROOTS PRIOR TO CANNING, CALCIUM HYDROXIDE CONCENTRATION AND LENGTH OF SOAK TIME, ON THE FIRMNESS OF CANNED SWEET POTATOES AS MEASURED BY THE ASCO FIRMNESS METER

Storage time (days)	Calcium hydroxide (wt) in water %	Soak time* (min)		ASCO firmness means
		3	6	
0	0	68.33 h	22.22 n	33.16
	2	39.00 l	17.56 p	
	4	21.22 q	30.67 m	
30	0	50.89 k	66.67 i	65.25
	2	80.11 d	66.44 i	
	4	74.00 f	53.44 j	
90	0	81.89 b	84.44 a	79.38
	2	80.33 d	81.22 c	
	4	77.00 e	71.44 g	
Means		63.64	54.90	

\* Figures not followed by common letters are significantly different at the 5 per cent level of probability.

centration could not be drawn without further analysis. However, the data showed a significant effect for pectin on firmness as measured by the ASCO firmness meter ( $F=6.80$ ).

The mean single treatment effects in terms of firmness are shown in Table 1 with the results of the Duncan multiple range test indicated. The higher ASCO firmness values indicate softer roots. The overall ASCO firmness means for zero storage time, 30 days storage and 90 days storage were 33.16, 65.25, and 79.38 respectively. These three means show the tendency of sweet potato roots to become softer as storage time increased. The evidence confirmed the fact that the constituents responsible for firmness of the product degraded during storage as suggested by McConnell<sup>1</sup>. The chemically degraded pectin apparently did not combine with calcium to form calcium-pectinate and thus was unable to produce an acceptable firmness.

The ASCO firmness values of individual treatments indicated that for the 3 min soak time there was a gradual decrease in ASCO firmness values as the per cent calcium increased, indicating that calcium treatment aided in firming sweet potato roots canned within 24 hr of digging or those stored prior to canning. This agrees with the conclusions reached by

Williams and Ammerman<sup>7</sup>. Lower ASCO firmness values (firmer roots) were obtained on an average for the 6 min soak (54.90) compared to the 3 min soak (63.64).

Analysis by the Duncan's New Multiple Range Test (Table 1) indicated that the treatment means for the roots stored for 30 days and soaked for 6 min in 0 and 2 per cent calcium hydroxide solution were not significantly different. The roots stored 90 days prior to canning, soaked 6 min in water with no added calcium were significantly softer than any other lot. Longer soaking time in the water with no calcium added resulted in significantly softer roots in most cases where the sweet potatoes had been stored longer than 24 hr after digging. Soaking the roots in water with increasing concentrations of calcium hydroxide from 0, 2 and 4 per cent resulted in significant increases in firmness of roots canned after 90 days storage for both the 3 and 6 min soaking times.

A study was conducted using sweet potato roots stored 90 days and canned with no calcium and no pectin treatment, to evaluate the effect of high temperature short time processing on firmness. The heat treatment of 132.2°C for 2 min at a reel speed of 5 rpm in the steritort resulted in roots with an average ASCO firmness value of 96.20 and a range for five cans of 83 to 100. Thus, the HTST process did not result in firmness comparable to the 6 min soak in a 4 per cent calcium hydroxide solution which was the best chemical treatment (71.44) for roots stored 90 days.

*Shear press results:* The average shear values for canned sweet potatoes in pounds of force as influenced by storage time, calcium concentration, and soak time are shown in Table 2. Analysis of variance indicated that the interactions for storage time *vs* calcium concentration and storage time *vs* soak time were highly significant.

The average shear value for zero storage time was 21.67 compared to 13.00 for 30 days and 11.18 for 90 days storage. The data show a consistent reduction in mean shear values with increasing storage time. This decrease in shear values as the storage time increased, supported the hypothesis that freshly canned roots were more resistant to shear than roots canned after 30 or 90 days storage.

The highest shear value was obtained for zero storage time of the raw roots, a 6 min soak time and a 2 per cent calcium solution (30.33), compared to the lowest shear value obtained for roots exposed to 90 days storage time, 0 per cent calcium and a 6 min oak (6.38). These values were significantly different

TABLE 2. CANNED SWEET POTATO SHEAR PRESS TREATMENT MEANS FOR TWO LEVELS OF SOAK TIME IN THE CALCIUM HYDROXIDE SOLUTION, THREE CONCENTRATIONS OF CALCIUM HYDROXIDE, AND THREE LEVELS OF STORAGE TIME OF THE ROOTS PRIOR TO CANNING

Storage time (days)	Calcium hydroxide wt. in water %	Soak time* min		Shear value means
		3	6	
0	0	14.20 cde	13.17 cde	21.67
	2	19.61 bc	30.33 a	
	4	23.94 ab	28.78 a	
30	0	9.94 de	9.89 de	13.00
	2	9.06 e	17.94 bc	
	4	11.78 cde	19.39 bc	
90	0	6.89 e	6.38 e	11.18
	2	11.39 cde	18.33 bc	
	4	12.11 cde	12.00 cde	

\* Means not followed by common letters are significantly different at the 5 per cent level of probability.

at the 5 per cent level of probability. Although there was a consistent trend toward higher shear values as the concentration of calcium hydroxide in the soaking solution increased the differences were generally not significant. The lack of significant differences in shear values due to the treatments in this study in which the ASCO values were much more reproducible and consistent, leads the authors to seriously question whether or not the shear values are indicators of firmness in the ordinary sense of the term.

*Pectin:* Analysis of variance showed that pectin concentration did not enter into any significant interaction, but pectin did have a highly significant effect on firmness as measured by ASCO firmness values ( $F=6.80$  \*\*).

At the higher pectin concentrations, the canned products were more firm as measured by the ASCO meter thus supporting the hypothesis developed by Williams and Ammerman<sup>7</sup>. The raw data for the three levels of pectin in the canning syrup, three storage times, three levels of calcium and two soak times are shown in Table 3. The data show that at 4 per cent calcium and 6 min soak time there is a consistent increase in ASCO firmness as the per cent pectin in the canning syrup increases.

TABLE 3. THE TREATMENT MEANS FOR ASCO METER VALUES, ROOT DIAMETER, AND SHEAR VALUES FOR SWEET POTATOES STORED 0, 30 AND 90 DAYS PRIOR TO PROCESSING, AND TREATED WITH THE INDICATED LEVELS OF PECTIN, CALCIUM, AND SOAK TIME

Pectin% wt	0% Ca (OH) <sub>2</sub> , 3 min soak			0% Ca (OH) <sub>2</sub> , 6 min soak			2% Ca (OH) <sub>2</sub> , 3 min soak			2% Ca (OH) <sub>2</sub> , 6 min soak			4% Ca (OH) <sub>2</sub> , 3 min soak			4% Ca (OH) <sub>2</sub> , 6 min soak		
	0	1.5	3	0	1.5	3	0	1.5	3	0	1.5	3	0	1.5	3	0	1.5	3
	0 day																	
Diam	4.04	3.58	3.31	3.30	3.37	3.24	3.40	3.67	3.77	3.10	3.00	3.40	3.24	3.74	3.17	4.07	3.87	3.37
ASCO	91.00	72.00	42.00	20.67	15.00	28.00	58.34	49.50	25.67	20.00	19.67	13.00	26.67	17.34	20.00	32.67	31.00	28.44
Shear	5.10	20.00	17.50	12.67	15.84	11.00	19.67	15.84	23.34	33.67	27.67	29.67	18.34	27.34	26.17	29.50	30.50	26.33
	30 days																	
Diam	3.90	3.44	3.77	4.24	4.14	3.80	3.67	3.64	3.30	4.20	4.20	3.77	4.04	4.44	3.74	4.07	4.30	4.37
ASCO	50.67	44.67	57.34	78.00	63.00	59.00	81.34	83.34	75.67	70.00	60.34	69.00	75.34	80.00	66.67	57.00	52.67	50.67
Shear	8.34	11.84	9.67	8.17	12.50	9.00	7.67	9.84	9.67	17.17	17.34	19.34	9.50	12.00	13.84	17.84	20.84	19.50
	90 days																	
Diam	3.44	3.90	4.45	4.03	4.40	3.97	4.10	4.57	4.07	4.07	3.94	4.27	4.10	3.80	4.40	4.24	4.04	3.77
ASCO	83.67	79.34	82.67	78.67	85.67	89.00	85.00	76.00	80.00	82.67	77.00	84.00	80.67	79.34	71.00	72.00	75.00	67.34
Shear	9.00	6.50	5.17	3.67	7.50	8.00	10.34	11.34	12.50	15.50	17.84	21.67	14.67	8.67	13.00	14.17	13.50	8.34

Pectin significantly influenced ASCO firmness values, but had no significant effect on shear values; therefore, these data also indicate that the parameters measured by the shear press and ASCO firmness meter were different. The ASCO firmness meter measured the force needed to bring about deformation of the roots in a given time or firmness, whereas the shear press was a measure of force in pounds to shear or cut through the product. There is no universally accepted definition of firmness, but it appears that the ASCO meter measures firmness while the shear press measures crispness or resistance to cutting or splitting.

Increased length of storage before canning resulted in a general increase in darkness of the canned roots as measured by Hunter 'L' values. The average Hunter 'L' value for roots canned with no storage was 41.49 compared to 39.80 and 39.87 for roots canned after 30 and 90 days of storage, respectively. An increase in soak time in the calcium hydroxide solution from 3 to 6 min resulted in a significant increase in darkness in 5 of our 6 tests for roots stored 30 days *vs* those canned the day they were harvested. For roots stored 90 days there was no significant difference in the darkness of the canned roots soaked 3 or 6 min in 0, 2 and 4 per cent calcium hydroxide solutions. Apparently darkening of the roots stored 90 days had progressed to such an extent that soaking had no further effect. There was a

general trend towards more darkness as the concentration of calcium hydroxide increased from 0 to 4 per cent by weight.

There was also a marked reduction in redness as measured by Hunter 'a' values with increased storage time from 0 through 90 days. Roots packed the day they were dug had a Hunter 'a' value of 21.05 compared to 18.55 and 18.06 for roots canned after 30 and 90 days of storage, respectively. Increased soak times from 3 to 6 min resulted in either no significant difference or a significant increase in redness. The average Hunter 'a' value for all roots soaked in calcium hydroxide solutions was 19.03 compared to the average value of 19.73 for the control roots.

Neither storage time nor length of soak had a practical effect on yellowness of the canned roots as measured by Hunter 'b' values. The average Hunter 'b' value for the roots canned the day they were harvested was 21.73 compared to 20.91 and 21.02 for the roots stored 30 and 90 days, respectively. The average Hunter 'b' value for the 3 min soak was 21.44 compared to 21.00 for the 6 min soak. There was a tendency for the Hunter 'b' values or degree of yellowness to be reduced by increasing concentrations of calcium hydroxide in the soaking solutions, but the differences were not of practical consequences.

Data on the shear values, ASCO firmness and colour data for 9 samples of commercially canned

sweet potatoes purchased from retail stores in Starkville, Mississippi were as follows :

	Commercial Average	sample Range
ASCO firmness	50.22	32.50-72.50
Root diameter	3.91	2.70- 4.70
Shear values	28.11	16.50-45.75
Hunter values		
L	44.51	39.20-51.05
a	15.96	13.65-20.00
b	20.77	17.55-23.45

Comparison of these data with the experimental data in Table 1 shows that calcium treated roots canned the day they were harvested are considerably more firm than the commercial samples. Roots canned after 30 days of storage were generally within the range of the commercial sweet potatoes, but were towards the soft end of the range. However, a 6 min soak in a 4 per cent calcium hydroxide solution resulted in roots with an average ASCO value of 53.44 when canned after 30 days of storage which was not much softer than the 50.22 average value for the commercial samples. After 90 days storage prior to canning only the roots soaked 6 min in the 4 per cent solution were in the commercial range with all other treatments resulting in substantially softer roots.

Data in Table 2 may be compared to the commercial shear press values. The roots canned the day they were dug which did not receive any calcium treatment

had less resistance to shear than the commercial sweet potatoes. However, treatment with calcium at either the 2 or 4 per cent concentration for either 3 or 6 min resulted in canned roots well within the commercial range. When roots had been stored 30 days only the 6 min soak resulted in firmness in the commercial range with 2 or 4 per cent calcium hydroxide solutions. After roots had been stored 90 days only the 6 min soak time resulted in commercial firmness.

When 1.5 or 3.0 per cent pectin was incorporated into the canning syrup the average ASCO firmness value was well within commercial range while the average shear values were outside the commercial range. The HTST process of 2 min at 132.2°C failed to produce canned sweet potatoes in the commercial range for either ASCO or shear values.

Use of pectin in the canning syrup resulted in Hunter 'L', 'a', and 'b' values within the commercial range.

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# Studies on the Insecticidal Activity of Garlic Oil. I. Differential Toxicity of the Oil to *Musca domestica nebulosa* Fabr and *Trogoderma granarium* Everts

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The relative toxicity of garlic oil to young, adults and mature larvae of housefly *Musca domestica nebulosa* Fabr and khapra beetle, *Trogoderma granarium* Everts has been investigated. The adults as well as the larvae of both the species respond to the vapours of garlic oil by exhibiting hyper-excitability, ataxia, salivation and excretion. In housefly adults, hyper-excitation was followed by ataxia within 4 to 6 min of exposure and 100 per cent mortality in 60 min. For khapra beetle, an exposure of 180 min was required to produce complete immobility and 100 per cent mortality. Although larvae of both the species were knocked down on exposure for 90 min, an exposure of 3 and 7 hr was necessary for 100 per cent mortality of housefly and khapra beetle larvae respectively. The adults which developed from the treated larvae were fertile. These observations indicate that the housefly is more susceptible to the toxic action of the oil than khapra beetle and that the adults of both the species are more susceptible than their larvae.

Although use of garlic, *Allium sativum* L. as a spice is well known, it is only recently that its oil has been shown to have larvicidal action against mosquitoes<sup>1,2</sup>, cabbage white butterfly and Colorado beetle<sup>3</sup>. Fairly low concentration (5 ppm) of both natural as well as synthetic garlic oil have been reported<sup>2</sup> to be effective in controlling mosquito larvae, and thus the oil appears to be as potent as some of the existing chemical insecticides such as DDT and BHC<sup>4</sup>. Since garlic is widely used for edible purpose, it is unlikely to be toxic to man. However, effectiveness of the oil as a wide spectrum pesticide needs to be established further. We have studied the effect of garlic oil on the larvae and adults of housefly *Musca domestica nebulosa* Fabr, a domestic pest, and khapra beetle, *Trogoderma granarium* Everts, a serious pest of stored grain products and the results are communicated in this paper.

## Material and Methods

Garlic oil, natural and synthetic, was obtained from Dr S. V. Amonkar of this institute.

Adult houseflies were maintained *ad libitum* on cane sugar, whole milk powder and water, while the larval stages on a standardized artificial medium consisting of yeast, agar and milk powder (4:1:5)<sup>5</sup>. A 16-day cycle from egg to egg, using the first batch of eggs laid by 4-day old females, was maintained at  $30 \pm 1^\circ\text{C}$  and  $60 \pm 5$  per cent R.H.

The khapra beetles were reared on semolina at  $34 \pm 1^\circ\text{C}$  and 50-60 per cent R. H. as described earlier<sup>6</sup>.

All the experiments were carried out using 1 to 2 day old adults, unless otherwise mentioned. Housefly larvae were 4-5 day old while those of khapra beetle 20-22 day old.

*Procedure for treatment:* Except for the toxicity studies with adult houseflies, where both natural as well as synthetic garlic oils were tested separately, in all other experiments only synthetic garlic oil was employed. One ml of the oil was taken in a 5 cm diameter petridish and kept inside a 2 lit capacity desiccator carefully sealed with grease. The chamber was used one hour after introduction of the oil in it. Insects of known age and stage were enclosed in a glass container  $5 \times 6.5$  cm with muslin and inverted over the petridish for exposure to vapours of the oil. Entire procedure was carried out at  $26 \pm 1^\circ\text{C}$  and the treated insects were returned to their respective diets specified earlier. Each experiment was replicated four times with 50 insects in each replicate. Any deviation from this procedure will be mentioned at appropriate places in the text.

The duration of exposure varied with the insects as well as the developmental stage. Adult houseflies were exposed for a period ranging from 5 to 60 min, while their larvae from 5 to 300 min. The exposure

time for the beetles ranged from 5 to 180 min, and their larvae from 5 to 420 min.

## Results

**Toxicity to adult insects:** Observations on the effect of garlic oil on the mortality of one to two day old adults of housefly and khapra beetle were made continuously for the first six-hour after treatment and thereafter once in 24 hr for six days and the results are presented in Fig. 1 and 2 respectively. The adults of both the species respond to the vapours of the oil by exhibiting hyperexcitability, followed by complete immobility or ataxia, salivation and excretion.

**Housefly:** Adults of housefly were generally knocked down within 4 to 6 min of exposure. Occasionally this period was as short as 2 to 3 min, but often extended to 14 min after exposure. Such variations were noticed irrespective of whether the oil was natural or synthetic, or from the same or a different batch, and were difficult to explain since the data were obtained under identical experimental conditions. Mortality was found to be intimately related to the initial period of knock down and data obtained from insects immobilized within 4 to 5 min of exposure to natural oil are presented in Fig. 1.

It will be noticed that on 60 min exposure, all the flies died without recovering from the toxic effect of garlic oil vapours. On exposure for 30 min, 80 per cent of the flies did not recover from the toxicants' effect while 20 per cent which survived, were sluggish and showed excessive salivation and excretion and died within three days. With further decrease in

exposure time to 20 min, the percentage of flies dying within first six-hour was also reduced to about 35. However, an additional 20 per cent died overnight, such that total mortality within a day was 55 per cent. Except for excessive salivation and excretion, the survivors looked normal and rate of their mortality was very low as even on 6th day of treatment 14 per cent flies were surviving. On 15 min exposure, although the percentage of flies unable to recover from ataxia was more or less same as that on 20 min exposure, the rate of mortality of the survivors was comparatively lower: only 50 per cent mortality was recorded by fifth day of the experiment. When flies were exposed for 10 min, 7 per cent succumbed to the toxicant during first six-hour and additional 7 per cent died overnight, thereafter the mortality was very low and even on 6th day after exposure it was only 45 per cent. On exposure to garlic oil for 5 min, the recovery was almost instantaneous and the flies showed no visible sign of the toxicity. In spite of this, about 6 per cent died overnight, thereafter, their mortality pattern was similar to that of the untreated control flies.

More or less similar results were obtained with flies upto 8 days of age. However, older flies (12 days and above) were more susceptible to the garlic oil e.g., on 15 min exposure, 50 per cent mortality was recorded within 24 hr of treatment. Treatment with synthetic oil also gave similar results.

**Khapra beetle:** Response of khapra beetle adults to garlic oil was similar to that of housefly in so far as hyperexcitability, ataxia, salivation and excretion were concerned. However, for knock down as well as for 100 per cent mortality, longer exposure of 180 min was required (Fig. 2) and the period of hyperexcitability also varied e.g., on 60 and 30 min exposure, hyperexcitability lasted for 4 to 5 hr after treatment. Moreover, mortality data obtained for adults on shorter exposures varied irrespective of the oil being from same or different batch e.g., comparison of data 24-hr after treatment for 100, 60, 30 and 15 min with garlic oil indicate that percentage mortality was 100, 90, 40 and 4 respectively (Fig. 2). In another set of experiments although 100 per cent mortality was recorded on 180 min exposure, the adults exposed to the oil for 60, 30 and 15 min showed 100 per cent survival even one week after exposure.

**Toxicity to larva:** Like adults, larvae also respond to the vapours of garlic oil by exhibiting hyperexcitability followed by ataxia, salivation and excretion. However, for both the test species, complete immobility was recorded only after prolonged exposure of 90 min and above.

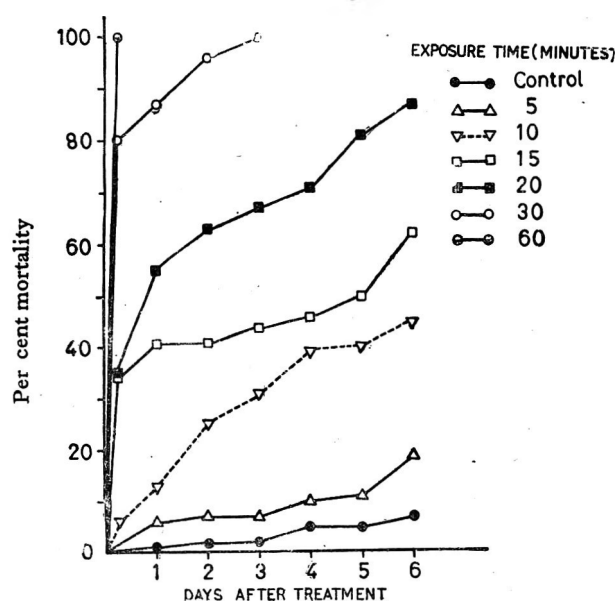


FIG. 1. Effect of garlic oil on adult *M. domestica*

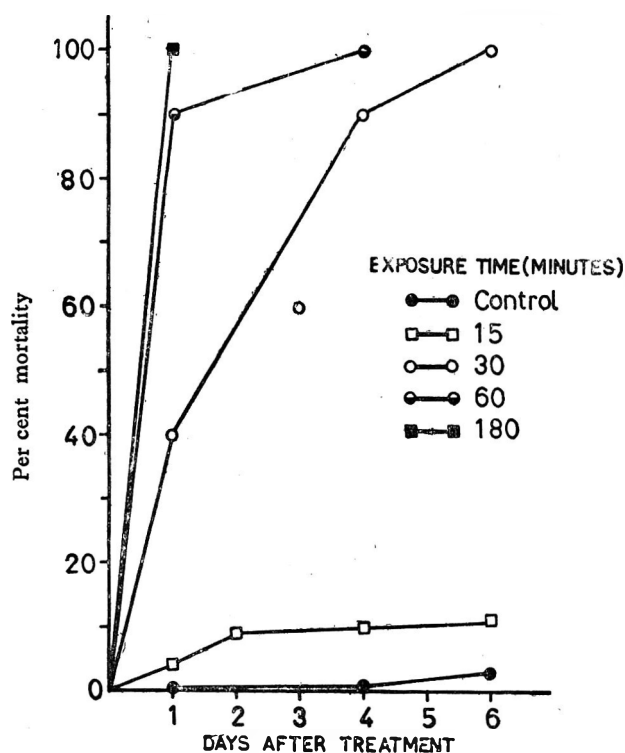


FIG. 2. Effect of garlic oil on adult *T. granarium*

**Housefly larva:** In case of housefly larvae (Fig. 3A), 180 min exposure was sufficient for 100 per cent kill within 24 hr, when all the larvae turned black in colour. Even exposure of 60 and 45 min were quite fatal as 88 and 81 per cent larvae respectively died overnight and the remaining which did pupate died without completing metamorphosis. With further decrease in exposure time from 30 to 5 min, larval mortality also declined from 39 to 2 per cent, and adult emergence increased from 11 to 98 per cent. Garlic oil did not affect the duration of larval period or fertility of adults emerged from the treated larvae.

**Khapra beetle larva:** In case of khapra beetle larvae (Fig. 3B) an exposure of 7 hr was necessary to get 100 per cent mortality in 48 hr. The larvae treated with the massive dose remained completely prostrated and turned brownish-black over a period of 48 hr when they were regarded to be dead. In spite of high larval mortality (65 and 70 per cent) on 300 and 100 min exposure, about 32 and 27 per cent larvae respectively developed into normal adults. On 1 hour exposure 96 per cent larvae were able to develop into adults. As in the case of housefly, adults developed from treated larvae were fertile.

## Discussion

During the course of studies on isolation and characterization of larvicidal principle of garlic<sup>2</sup>, the oil was

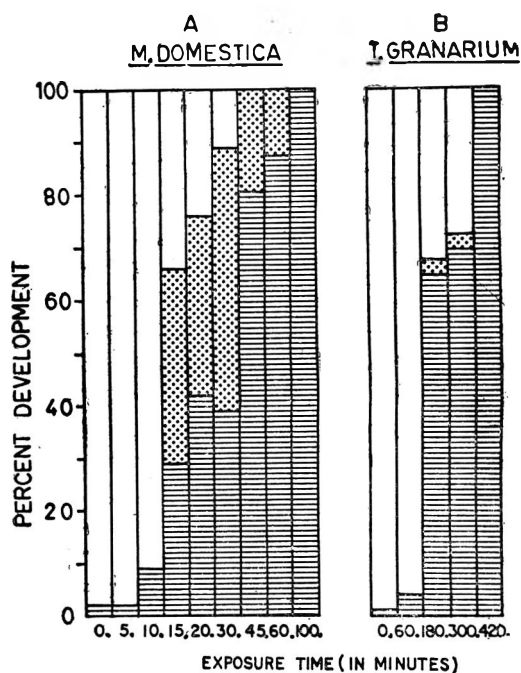


Fig. 3 EFFECT OF GARLIC OIL ON THE LARVA OF *M. DOMESTICA* & *T. GRANARIUM*

ADULT PUPA LARVA A: 7 Days after treatment  
B: 15 Days after treatment

also reported to have 'antagonistic' properties towards potato tuber moth, red cotton bug, red palm weevil, houseflies and mosquitoes. However, the nature of 'antagonism' or relative toxicity of the oil to these insects was not described. Present studies indicate that adults as well as larvae of both housefly and khapra beetle respond to the garlic oil vapours by exhibiting hyperexcitability followed by ataxia, salivation and excretion. The symptoms of poisoning suggest that like pyrethrins, DDT and organophosphates<sup>7,8</sup> the major action of the oil may either be neuromuscular blockage or metabolic inhibition.

Differences in the manifestations of symptoms of poisoning from few minutes after exposure in the case of housefly adults to about 90 min or more in its larvae and khapra beetle, further suggest that the effect of the oil is highly species and stage specific. Housefly is more susceptible to the oil than khapra beetle and the adults of both the species are more susceptible than their larvae. It is likely that these differences may be due to the differences in one or more steps<sup>4</sup> such as, the degree of penetration of garlic oil vapours into the insects, its metabolism, storage and excretion, penetration into the target, and attack upon the target. Both housefly and khapra beetle appear to be less susceptible than the various



species of mosquitoes, which are killed by as low as 3.5 to 9.4 ppm of garlic oil<sup>1,2</sup>. However, these insects were found to be good test organisms for studying the mode of action of garlic oil as an insecticide<sup>3</sup>.

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# Hypocholesterolemic Effect of Sardine Oil and Oil-sardine (*Sardinella longiceps*) Fish

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**In cholesterol bile-salt stressed rats, sardine oil or oil-sardine fish reduced serum cholesterol. In the absence of dietary added cholesterol, these had no effect on serum cholesterol. Sardine oil, but not sardine fish, seemed to depress the growth of rats significantly. The weights of heart and liver of rats grown on diets containing sardine oil/sardine fish with or without cholesterol were not significantly different. Sardine oil or oil-sardine did not have any significant effect on cholesterol content of heart or liver. Histopathologically, however, in rats fed cholesterol, but not sardine oil or sardine fish, fat cysts were present in liver.**

Polyunsaturated (vegetable) oils have been found to lower cholesterol and phospholipid levels of patients affected with various metabolic disturbances.<sup>1-3</sup> During the last two decades, the efficacy of polyunsaturated marine oils (liver oils of cod, halibut, dog fish and cuttle fish, and body oils of menhaden, tuna, salmon, mullet, herring and whale) in reducing serum cholesterol level in man, chicken, mice and rats<sup>4</sup> have been established. Hypocholesterolemic effect of fish in the diet of rats and human has been reported by Kinsell,<sup>5</sup> Miller, *et al.*<sup>6</sup> and Harlow and Morton.<sup>7</sup>

Oil-sardine (*Sardinella longiceps*) which is a fatty fish with maximum fat content of 11-16 per cent during season is the most important fishery in our country.<sup>8-10</sup> Sardine oil with high content of polyenoic acids (particularly pentaenoic and hexaenoic

acids)<sup>11</sup> and low cholesterol content<sup>12,13</sup> is the only marine oil of commercial importance in India.

With a view to find out certain specific dietary advantage of oil-sardine fish and an additional avenue for the outlet of fish oil, it was considered worthwhile to study the possible hypocholesterolemic effect of these materials in experimental hypercholesterolemia.

## Materials and Methods

Six week adult male rats of Wistar strain were fed a stock diet which contained about 14 per cent hydrogenated vegetable oil (HVO), 0.5 per cent cholesterol and 0.5 per cent bile salt (diet of Group I, Table 1), for a period of 4 weeks (pre-test period) to ensure severe hypercholesterolemia. To test the effect on endogenous cholesterol *per se*, another lot of rats was maintained on similar basal diet without cholesterol

TABLE 1. COMPOSITION OF TEST DIETS

	Gr I %	Gr II %	Gr III %	Gr IV %	Gr V %	Gr VI %
Casein	18.0	18.0	8.7	18.0	8.7	18.0
Sucrose	59.0	59.0	59.0	59.0	59.0	59.0
HVO	13.9	3.9	3.9 <sup>a</sup>	3.9	3.9 <sup>a</sup>	13.9
RGO	1.0	1.0	1.0	1.0	1.0	1.0
Cellulose	4.0	4.0	2.4	5.0	3.4	5.0
Salt mix <sup>†</sup>	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin mix <sup>‡</sup>	1.0	1.0	1.0	1.0	1.0	1.0
Vitaminised oil <sup>b</sup>	0.1	0.1	0.1	0.1	0.1	0.1
Cholesterol	0.5	0.5	0.5	—	—	—
Bile salt	0.5	0.5	0.5	—	—	—
Sardine oil <sup>c</sup>	—	10.0	—	10.0	—	—
Oil-sardine fish	—	—	53g/79.1g of diet	—	53g/79.1g of diet	—

a = Contained added alpha-tocopherol acetate (190 mg/100g).

b = Containing vitamin A (2000 I.U.), D (1000 I.U.), and E (100 I.U.) per g oil.

c = FFA, 0.18% as oleic acid; Iodine value (Wijs) = 166.7; added alpha-tocopherol acetate, 75 mg/100 g.

Calorie derived from protein, 16.2%; fat, 30.3% and carbohydrate, 53.5%.

HVO: Hydrogenated vegetable oil; RGO: Refined groundnut oil.

and bile salt (diet of Group VI, Table 1). These were then divided into six groups of 8 rats each by randomized block design and were fed test diets (Table 1) for a period of 45-55 days; in test lipid diets (Groups II, III, IV and V), sardine oil either as such or that derived from oil-sardine fish, replaced 66.7 per cent of HVO and refined groundnut oil (GNO) taken together. Pre-test and test diets were essentially similar to those described by Peifer.<sup>14</sup>

For groups II, III, IV and V, stock diets without fish or oil were prepared weekly. Daily requisite quantity of sardine fish from a canned pack or sardine oil was mixed with corresponding stock diets and given at 20 g (dry weight) per rat. For the remaining groups, complete stock diets prepared weekly were given.

Sardine oil prepared from whole fish in the laboratory was used as such without further refining or processing. The oil had a free fatty acid content of 0.18 per cent as oleic acid and iodine value (Wijs) of 166.7. In order that there is an appropriate balance between polyenoic acids of fish oil and antioxidants, sardine oil was mixed with alphatocopherol acetate (75 mg/100 g oil).<sup>15</sup> To prevent excessive oxidation

in handling and storage during the course of experiment, oil was bottled completely full in 100 ml amber coloured glass bottle and stored in a refrigerator. Content of each bottle was sufficient to meet 2-3 days requirements.

Sardine fish in the form of canned packs was used. Fresh catch of sardine fish was dressed, cut to size, washed and brined in saturated brine, packed in can and processed. The fish thus prepared had moisture, 60.5; protein (N × 6.25), 17.5; fat, 19.0; and ash, 3.1 per cent and was mixed with stock diet containing other components daily (53 g of fish mince was found to contain 10 g of fish oil, 9.3 g protein and 1.6 g of ash totalling 20.9 g solid matter and was mixed with 79.1 g of stock diet). Casein content of diets of groups III and IV given sardine fish was reduced to 8.7 g in view of contribution of protein by fish. To account for 1.6 g of mineral contents derived from fish, cellulose content was reduced proportionately. To meet the additional requirement of antioxidant on account of fish-oil derived from fish, HVO of the groups given sardine fish was mixed with alpha-tocopherol acetate (190 mg/100 g).

At the end of test period, rats were anaesthetized with diethyl ether and blood collected from heart. Liver and heart were lightly washed in a normal saline, wiped with filter paper to free them from extraneous water and weighed. A portion each of heart and liver was kept separately for estimation of cholesterol. Remaining portions along with entire viscera were kept in 4 per cent formalin for subsequent histopathological study.

Serum total cholesterol was estimated by developing colour by Liebermann-Burchards' reaction as described by Sackett.<sup>16</sup> Serum ester cholesterol was estimated by the method described by Bloor *et al.*<sup>17</sup> Difference in the figures for total and ester cholesterol was taken as serum free cholesterol. Serum phospholipid was estimated by the method described by Zilversmit and Davis.<sup>18</sup>

Weighted quantity of heart and liver was homogenised with a mixture of acetone-ethanol (1:1) and centrifuged. The residue was extracted twice. The extract was made up to a volume. Total cholesterol (heart and liver) and ester cholesterol (liver only) were estimated in aliquot portions of the extract by the methods referred to earlier.

## Results and Discussion

*Body weight and food intake:* In all groups excluding group IV, increase in body weight during test period was not statistically significant with reference to

the corresponding control (Table 2). Group IV fed with sardine oil without cholesterol had increase in body weight significantly lower than that observed with other groups. Group II was also fed sardine oil but along with cholesterol, the increase in body weight was comparable to control and other groups. Decreased growth rate has been reported when marine oil fed is not sufficiently stabilised and level of

peroxides are high.<sup>10</sup> Sardine oil used might have accumulated peroxide due to handling during the course of investigation and exhibited growth inhibitory effect. Investigation in this respect is being pursued. Apparently, exogenous supply of cholesterol along with fish oil counteracted this adverse effect and the observation is worth mentioning.

*Terminal serum cholesterol and phospholipids:* In cholesterol and bile salt stressed rats (Groups I, II and III), sardine oil and sardine fish in the diet lowered the terminal serum total, free and ester cholesterol Table 3. The lowering in total and ester cholesterol was statistically very highly significant and that for free cholesterol, highly significant. With sardine oil (Group II), serum total cholesterol was only 134 mg per cent as against 322 for control group (I). With sardine fish in the diet (III), corresponding figure was 120. Terminal ester cholesterol in control group (I) was 242 mg per cent; it was only 85 mg per cent in sardine oil group (II) and 71 mg per cent in sardine fish group (III). Terminal free cholesterol in control group (I) was 87 mg per cent, corresponding figure for sardine oil and sardine fish groups (II and III) was 50 mg per cent. No significant difference in terminal serum phospholipid content was observed in these groups. But total cholesterol/phospholipid ratio was lower in sardine oil and sardine fish groups (II, III) than that in control group (I) (2.80 for control group

TABLE 2. INCREASE IN BODY WEIGHT AND FOOD INTAKE DURING TEST PERIOD

Gr. No.	Diet	Av body wt. during test period (g)				Food intake (g/rat/day)*
		Pre-test	Beginning	End	Increase	
I. Control		155.2	235	316.2	81.2	12.7
II. Sardine oil		153.0	226	310.5	84.5	12.6
III. Sardine fish		152.2	225	321.6	96.6	12.3
IV. Sardine oil		152.4	229	289.4	60.4	12.1
V. Sardine fish		151.6	227	316.4	89.4	12.5
VI. Control		149.7	223	305.1	82.1	13.0
Standard error					7.2	0.18
Degrees of freedom					35	35

\* Moisture free basis; Test period=45-55 days.

TABLE 3. TERMINAL SERUM TOTAL, FREE AND ESTER CHOLESTEROL AND PHOSPHOLIPIDS (8 rats per group)

Gr. No.	Diet	Av. total cholesterol		Av. free cholesterol		Av. ester cholesterol mg/100 ml (EC)	Serum phospholipids mg/100 ml (PL) mean±S.E.	TC/PL mean±S.E.	FC/EC* mean	
		mg/100 ml (TC)	Transformed variate† mean±S.E.	mg/100 ml (FC)	Transformed variate† mean					
I. Control with cholesterol		322	2.50±0.036	87	1.92	242	119 ±12.6	2.80 ±0.09	0.44	
II. S.O. with cholesterol		134	2.12 „	50	1.66	85	132 ±12.6	1.04 ±0.09	0.56	
III. S.F. with cholesterol		120	2.97 „	50	1.66	71	138 ±12.6	0.90 ±0.09	0.82	
IV. S.O. without cholesterol		68	1.82 „	34	1.51	34	145†±13.6	0.51†±0.10	1.07	
V. S.F. without cholesterol		63	1.79 „	31	1.48	33	112 ±12.6	0.62 ±0.09	1.04	
VI. Control without cholesterol		70	1.83 „	38	1.55	32	146 ±12.6	0.51 ±0.09	1.16	
Standard deviation					0.156	28			0.44	
Degrees of freedom					(35)	(30)	(30)	(34)	(34)	(30)

† As serum cholesterol (both total and free) follows a log. normal distribution, analysis of variance has been carried out on the transformed variates using logarithmic transformation.

\* Serum free cholesterol (FC), ester cholesterol (EC) and FC/EC could not be obtained for 3 rats in diet I and one rat each in diet II and IV. Hence the values were obtained by the missing plot technique.

† Treatment mean containing a missing value.

S.O. : Sardine oil; S.F. : Sardine fish.

as against 1.04 and 0.90 for the other two groups) and the difference between control and treated groups was statistically very highly significant.

In the study of cholesterol content of serum or liver the distribution is not normal and therefore statistical analysis was carried out on the transformed variates.

To test the effect of sardine oil or sardine fish on endogenous serum cholesterol *per se*, rats of group IV, V and VI were maintained on diets without cholesterol. Contribution of cholesterol to the diet by sardine oil (cholesterol content, 0.4 per cent<sup>19</sup>) or sardine fish (cholesterol content 0.11 per cent<sup>20</sup>) was negligible (not more than 0.05 per cent). Terminal serum total cholesterol in control group (VI) was only 70 mg per cent and with sardine oil (IV) and sardine fish (V) it was 68 and 63 mg per cent respectively. These differences are not statistically significant. Thus apparently, sardine oil or sardine fish had no effect on endogenous synthesis of cholesterol, since serum level which reflects endogenous synthesis in the absence of exogenous supply was not significantly affected.

*Heart and liver weights:* Neither cholesterol in the diet nor sardine oil or sardine fish had any significant effect on heart weight either as such or on body weight basis (Table 4). Addition of cholesterol in the diet increased the liver weight either as such or

TABLE 4. AVERAGE TERMINAL LIVER AND HEART WEIGHTS

Gr. No.	Diet	Liver wt (g)		Heart wt (g)	
		Total	per 100 g body wt.	Total	per 100 g body wt.
I.	Control	14.1	4.52	1.35	0.45
II.	Sardine oil	13.9	4.52	1.46	0.47
III.	Sardine fish	14.5	4.58	1.47	0.46
IV.	Sardine oil	9.7	3.48	1.30	0.45
V.	Sardine fish	10.8	3.39	1.41	0.45
VI.	Control	10.5	3.49	1.33	0.44
S. E. of mean		±0.99	±0.25	±0.09	±0.026
Degree of freedom		(25)	(25)	(30)	(30)
Diet composition see Table 1.					

per gram body weight. Sardine oil or sardine fish had no effect on the liver weight when fed with or without cholesterol.

*Heart and liver cholesterol:* Cholesterol (total, free and ester) content of liver or total cholesterol content of heart was significantly higher in groups given cholesterol in the diet than that observed in other groups (Table 5). Effect of sardine oil or sardine fish on liver/heart total cholesterol content was not statistically significant.

TABLE 5. TERMINAL CHOLESTEROL CONTENT OF LIVER AND HEART

Group No.	Diet	Av. liver total chol*		Av. liver free chol*		Av. liver ester chol mg/g fresh tissue†	Av. heart total chol mg/g fresh tissue
		FTM mg/g	TVM	FTM mg/g	TVM		
I.	Control with cholesterol	34.41	1.4795	11.64	0.9876	24.54	3.10
II.	S.O. with cholesterol	34.09	1.4949	8.30	0.8880	27.89	2.89
III.	S.F. with cholesterol	28.04	1.4352	8.21	0.8727	20.48	3.00
IV.	S.F. without cholesterol	5.75	0.7499	2.47	0.3379	3.35	2.12
V.	S.O. without cholesterol	6.76	0.7728	3.77	0.5371	4.39	1.77
VI.	Control without cholesterol	4.42	0.6148	2.23	0.2851	2.32	1.90
Standard deviation		—	0.1684	—	0.2420	8.721	0.650
Degrees of freedom		—	(32)	—	(31)	(27)	(27)

\* As liver total cholesterol and liver free cholesterol follow a logarithmic distribution, statistical analysis has been carried out by using logarithmic transformation of the variates.

For one rat in each of the diets I, II and V liver total cholesterol could not be obtained. Similarly for one rat each of the diet I and II and for two rats in diet V liver free cholesterol for one rat in each of diets I, II and V, liver ester cholesterol, and for 3 rats in diet I, 2 rats each in diets II and III and one rat in diet V, heart total cholesterol could not be estimated. Hence these have been estimated by the missing plot technique.

† This contained only 7 rats/group; all other groups had 8 rats.

FTM: Fresh tissue mean; TVM: Transformed variate mean.

*Histopathological study of liver and aorta:* The livers of rats of Groups I, II and III showed similar picture. Liver cells showed fatty infiltration with normal (i.e., without disarray) of lobular architecture. However, fatty infiltration was more severe in the controlubular zone, where the liver cells showed large single vacuole and a peripherally situated nucleus. In the peripheral zones, the liver cells showed fine vacuolation of the cytoplasm and centrally placed nucleus. The tissue was positive for neutral fat and cholesterol. However, with livers of Group III given sardine fish, it was weakly positive for cholesterol. In rats fed cholesterol (Group I), fat cysts were present in liver but these were not observed in group given sardine oil (Group II) or oil-sardine fish (Group III).

Above histopathological study of livers of rats fed cholesterol indicates certain positive beneficial effect (not to the extent of restoring normal condition) of sardine oil and oil sardine fish, the effect of oil-sardine fish being more.

All the livers of rats of Group IV, V and VI had similar appearances. Liver architecture was normal. There was no fatty infiltration and distribution of neutral fat and cholesterol was normal.

Aorta was examined for possible atherosclerosis which was not observed in any group including the control groups. No abnormality was observed in groups fed sardine oil and sardine fish.

Under these series of experiments, four different and positive responses have been fully established in hypercholesterolemic rats fed sardine oil and sardine fish: (a) hypocholesterolemic response, (b) reduction in serum ester cholesterol level, (c) reduction in TC/PL ratio (all these responses were very highly significant), and (d) positive beneficial effect on histopathology of livers (effect of sardine fish being more).

Observation made with sardine fish requires special emphasis. Effect of sardine oil as such or in the form of fish was the same. Yet from the standpoint of practical application of the study the observation made with sardine fish is more important. Palatability and stability of sardine oil may offer certain difficulty in the matter of administration and nutrition but with sardine fish the problem does not arise. The study opens the possibility of formulating a diet based on sardine fish in controlling hypercholesterolemia. As the effect is due to fat content of fish, only fatty fish, i.e., those caught during September to January will show its

effect to a greater extent. Thus canned pack of fish with high fat content is expected to have additional advantage.

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# Studies on Chilled Storage of Fresh Water Fish. II. Factors Affecting Quality

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Some of the factors which effect the quality of chilled fresh fish during handling, storage and transportation were studied in the case of Indian fresh water fish species which are commercially important. It was observed that differences in species or size of fish did not significantly affect storage behaviour. Delay in chilling until the resolution of *rigor mortis* did not adversely affect quality on subsequent storage, but further delay did affect life. Suitability of chill air as a storage medium was subject to relative humidity, too low of which leading to dehydration and too high causing excessive surface bacterial growth. Storage in chilled water or melting ice prevented dehydration and lessened bacterial growth but appeared to cause slight flavour losses due to leaching. The increase in spoilage rate due to rise in storage temperature was less as compared to most marine species. Damage of tissues as a result of rough handling accelerated spoilage.

Storage behaviour of *Mrigal* (*Cirrhina mrigala*), a major carp, in ice, has been reported earlier.<sup>1</sup> An important observation based on organoleptic, chemical and bacteriological indices of spoilage was that these species have shown a relatively longer shelf life under ideally iced condition as compared to most marine species. Carps occupy an important place in the fresh fish trade in India and hence the above observation is of some significance from the point of view of storage and transportation. But it is necessary to study a number of other variables which influence the quality of chill stored fish in order to effect improvements in the handling, transportation and marketing. For instance the influence of species differences and variations in size is of practical importance in packaging and storage. It is also necessary to study how far such factors like delayed chilling and variations in temperature which are encountered in actual trade practices affect the quality of the fish. Data regarding the storage behaviour under different chilling media will also be useful for attempting alternatives in refrigerated transport.

## Materials and Methods

Major carps viz., *Catla catla*, *Labeo rohita*, *Cirrhina mrigala* and *Labeo calbasu* and the locally abundant *Barbus carnaticus* were used as raw materials. The experimental samples were procured from the reservoirs and tanks in and around Mysore city. Experimental details for the organoleptic, chemical

and microbiological tests employed during the storage period were the same as has been reported earlier.<sup>1</sup>

*Influence of size of fish on storage life:* In each species, fishes ranging in size from 1.5 to 6 kg (75 per cent marketed fall in this range<sup>2</sup>) were kept in crushed ice and stored in a cold room at 0-1°C.

*Storage life of different species:* Fishes belonging to the different species were stored in ice and examined periodically as described above. To minimize other variables, fishes of nearly same size were used.

*Effect of delayed chilling:* Freshly caught and slaughtered fishes belonging to a single species were used. One set of the samples was immediately iced. Other sets were exposed to room temperature (28-30°C) for varying periods before they were iced. Samples from each set were examined periodically.

*Influence of the medium of chilling:* The three conventional chilling media viz., cold air, ice and chilled water were used. In the case of air chilling, the fish were kept in a cold chamber at 0-1°C and 40 per cent RH and another at the same temperature but with a device to maintain the RH at about 90 per cent. A third set of sample was kept in chilled water at 0°C and the fourth set was stored in crushed ice. (The chilled water was maintained at 0°C by keeping excess of crushed ice in it).

*Variations in storage temperature:* Separate sets of fish samples were stored in three chambers maintained at 0, 5 and 10°C, with a uniform humidity of 35-40 per cent RH.

**Physical damage to flesh and rate of spoilage:** In the same batch of fish, samples with damaged tissues (caused by rough handling) were separated from unaffected ones. Both were stored in ice under same conditions, and compared at definite intervals. Also, from the same fish, damaged flesh portions were compared with unaffected parts for microbial growth rate and other spoilage indices.

In all the above experiments, fishes of the same species of comparable size were used.

## Results and Discussion

Statistics collected from a major marketing centre in India<sup>2</sup> show distinct differences in the quality of marketed fish depending on size. Accordingly, the size and percentage of iced fish becoming sub-standard on arrival in Howrah are as follows: Below 1 kg, 90 per cent; 1 to 3 kg, 50 per cent and above 3 kg, 20-30 per cent. But in the present case (Table 1) the extent of spoilage appears to be practically the same for all fishes ranging in size from 1.6 to 6.1 kg comprising two important species, at the end of 10 days storage. This is reasonably a long period for ice-stored fish to have exhibited any significant differences in the rate of spoilage. All the samples were organoleptically good at the end of the storage period.

Species differences, on the other hand, are known to influence storage behaviour among marine fishes. Bramsnaes<sup>3</sup> has summarised the storage life of different species of North Atlantic fish in ice; it ranged from 9 days for whiting to 21 days for halibut. But no such differences are evident among the five major commer-

TABLE 2. STORAGE BEHAVIOUR OF DIFFERENT SPECIES

Species	No.	Organoleptic score		TVB mg%	TBC/g
		Raw	Cooked		
<i>Labeo rohita</i> (Rohu)	1	7.7	8.1	13.4	$6.2 \times 10^6$
	2	8.2	8.4	12.3	—
	3	8.1	8.5	12.8	—
<i>Cirrhina mrigala</i> (Mrigal)	1	8.2	8.6	12.6	$1.0 \times 10^6$
	2	7.8	8.1	12.1	—
	3	8.2	8.5	13.0	—
<i>Labeo calbasu</i> (Calbasu)	1	8.1	8.5	13.4	$5.0 \times 10^6$
	2	7.9	8.1	12.8	—
	3	8.2	8.4	13.7	—
<i>Barbus carnaticus</i> (Gende)	1	7.9	8.3	11.9	$3.3 \times 10^5$
	2	8.1	8.0	12.3	—
	3	8.4	8.2	13.0	—
<i>Catla catla</i> (Catla)	1	8.2	8.6	13.0	$8.3 \times 10^6$
	2	7.9	8.2	12.1	—

Storage period, 10 days  
TBC: Total bacterial count

cial species of fresh water fish compared in the present study (Table 2). But it may be mentioned that Bramsnaes' list represents a wide cross section of marine food fishes vastly varying in body composition, feeding habits, habitat, etc., which define biochemical and bacteriological factors responsible for spoilage rate. Such variations being minimum in the species of fresh water fish considered here,<sup>1</sup> the observed similarity in storage behaviour seems possible. Again, this observation belies popular belief that some species like *mrigal* spoil faster than others. Perhaps this aspect needs further critical study keeping in view the variable ambient temperature. However, it may be concluded that under ideally iced condition the storage behaviour is alike for all the species considered for the period of observation and all remained acceptable at the end.

Exposure after death to higher temperatures prior to icing is reported to affect adversely subsequent storage life of marine fish, the extent of such spoilage depending on the extent of delay in icing<sup>4-6</sup>. Due to the widespread use of gill nets in inland fisheries in India, there is an uncertain time-lag between death of the fish (which depends on the time the fish gets entangled in the net) and removal from water. This time lag may vary from 0 to 8 hr in actual practice. There is a further delay of a few hours before icing, making the overall time lapse between death and icing about 6 to 14 hr<sup>2</sup>. Normally, this length of period of exposure to higher temperature is sufficient to shorten subsequent storage life, since the period is sufficient to boost the initial bacterial population to appreciable levels. Under

TABLE 1. INFLUENCE OF SIZE ON QUALITY OF ICE-STORED FISH

Species	Size (kg)	Organoleptic score		TVB mg%	Total bacterial count/g
		Raw	Cooked		
<i>Cirrhina mrigala</i> (Mrigal)	1.6	7.9	8.1	12.3	$2.8 \times 10^5$
	1.8	8.2	8.3	12.6	—
	1.8	7.8	8.0	11.9	—
	2.1	7.8	8.0	12.5	$7.9 \times 10^5$
	2.7	8.1	8.3	13.0	—
	3.0	7.9	8.2	12.8	—
	3.7	8.1	8.2	12.0	$1.1 \times 10^6$
	3.9	8.0	8.3	12.1	—
	4.2	7.8	8.0	12.6	$6.9 \times 10^5$
	5.4	8.1	8.2	12.0	—
<i>Labeo rohita</i> (Rohu)	1.7	7.8	8.1	13.0	$2.6 \times 10^5$
	1.9	8.1	8.4	11.9	—
	2.7	8.0	8.1	12.8	$5.9 \times 10^5$
	3.0	7.7	7.9	13.2	—
	4.9	7.9	8.2	12.8	$4.7 \times 10^5$
	6.1	8.1	8.3	11.8	—

Fish becomes inedible when the score falls below 4. Storage period: 10 days.

the controlled conditions in the present study, a lapse of 7 hr before icing does not appear to have any adverse effect on storage life (Table 3). Any further delay beyond 7 hr in icing shows distinct deleterious effects later. It was observed that the first 7 hr approximately correspond to the total time taken for onset, duration and resolution of *rigor mortis* as judged by physical examination. The rigor period is known to be unfavourable for spoilage bacteria in fish<sup>8,9</sup>. Hence, rapid growth of the latter commences only after the resolution of the rigor. The observed stability of fish iced during the rigor period and the progressively reduced shelf life of fish iced afterwards are attributable to the level of bacterial load on the fish immediately prior to chilling, which in turn, is a consequence of the above mentioned phenomenon. In this context, the larger spoilage incidence in smaller fishes observed in Howrah<sup>2</sup> referred earlier, may find some explanation. According to Amlacher<sup>10</sup>, the period of onset and duration of *rigor mortis* is shorter in smaller specimens in the same species of fish. In the prevailing practice of inland water fishing with the usual delay in icing mentioned earlier, it is quite

probable that most of the smaller specimens have already passed rigor period much before the time of icing while most of the larger specimens were still in rigor.

Though icing is still recognised as the best medium of chilling fish, chilled water can be advantageously used in certain cases of large scale handling.<sup>7</sup> Similarly, though air cooling is not favourable in terms of rate of heat transfer, in long distance transportation of fish, a refrigerated chamber avoiding ice will be of much advantage. The capacity for retaining the freshness of fish appears to be the same in the case of both melting ice and chilled water at 0°C (Table 4). The superiority of ice over chilled water is that the former possesses, weight for weight, additional cooling capacity by virtue of its heat of fusion. This is obviously of advantage in transportation. But in large scale handling, especially on board fishing vessels, where mechanical refrigeration system for circulation of chilled water or brine can be installed, the latter offers a better system of chilling from the point of view of ease of handling and also faster cooling. But the behaviour of fish stored in cold air at 0-1°C is unique

TABLE 3. EFFECT OF DELAYED CHILLING ON QUALITY OF ICE-STORED FISH

Time between death and icing (hr)	After 7 days in ice				After 15 days in ice			
	Organoleptic score		TVB mg%	TBC per g*	Organoleptic score		TVB mg %	TBC per g*
	Raw	Cooked			Raw	Cooked		
0	8.0	8.3	11.1	4.3×10 <sup>4</sup>	8.0	7.6	12.7	3.7×10 <sup>5</sup>
4	7.9	8.1	10.9	7.2×10 <sup>4</sup>	7.4	7.7	12.4	1.5×10 <sup>5</sup>
7	8.1	8.2	11.3	1.8×10 <sup>5</sup>	7.9	8.0	13.0	7.2×10 <sup>5</sup>
9	7.6	7.9	12.4	3.3×10 <sup>5</sup>	6.9	7.1	13.9	6.8×10 <sup>5</sup>
12	6.9	7.2	13.1	6.3×10 <sup>5</sup>	6.1	6.4	18.1	3.4×10 <sup>6</sup>

\* Refers to one sample from each group.

TBC: Total bacterial count.

TABLE 4. INFLUENCE OF CHILLING MEDIUM ON STORAGE LIFE OF FISH

Storage method	After 7 days					After 15 days				
	Organoleptic score		TVB mg %	TBC per g.	Wt loss %	Organoleptic score		TVB mg %	TBC per g.	Wt loss %
	Raw	Cooked				Raw	Cooked			
Ice (melting)	8.3	8.4	10.3	4.3×10 <sup>4</sup>	0	8.0	8.1	12.2	3.9×10 <sup>5</sup>	0
Chilled water (0°C)	8.1	8.4	9.9	7.1×10 <sup>4</sup>	0	7.9	7.4	11.5	4.4×10 <sup>5</sup>	0
Air (0-1°C; 40% R.H.)	7.9	8.9	13.4	3.2×10 <sup>5</sup>	4.3	7.2	8.4	18.4	8.2×10 <sup>5</sup>	6.7
Air (0-1°C; 90% R.H.)	7.2	7.1	14.0	4.4×10 <sup>5</sup>	0	4.0*	...	24.9	5.4×10 <sup>7</sup>	0

\*A thick cover of bacterial slime.

TBC: Total bacterial count.



in many respects. Firstly, humidity becomes one important factor in this mode of storage. At low humidity, there is some loss of weight, and the organoleptic score (raw) is low apparently due to the dehydrated and shrunken appearance. At high humidity (about 90 per cent RH), though there is no loss of weight, the score is much lower. There was a thick layer of bacterial slime formed over the fish and hence putrefaction started early. This necessitates controlled conditions of humidity in air-chilling. The slightly higher cooked score in the case of air-chilled fish (40 per cent RH) over either of the ice-chilled or water-chilled samples, and the comparatively higher TVB values in the former are noteworthy. It is reasonable to assume that part of the flavouring components, which are water soluble, have been leached out from the flesh by melting ice. The volatile bases, which too are soluble in water might have been partially removed by the same process.

Differences in the rate of spoilage due to variations in storage temperatures above  $0^{\circ}\text{C}$  are shown in Fig. 1-3. Undoubtedly there is increased spoilage rate as the storage temperature is raised. To reach a score of 5.0 (raw), fish stored at 0, 5 and  $10^{\circ}\text{C}$  took storage periods of 27, 16 and 10 days respectively. These approximately correspond to storage periods which will take the TVB values to 25 mg per cent or the bacterial load to  $10^6/\text{g}$  in all cases. Thus it may be roughly taken that fresh water fish spoils at about 1.6 times as fast at  $5^{\circ}\text{C}$  and 2.7 times as fast at  $10^{\circ}\text{C}$  as it does at  $0^{\circ}\text{C}$ . This may be compared to figures quoted by

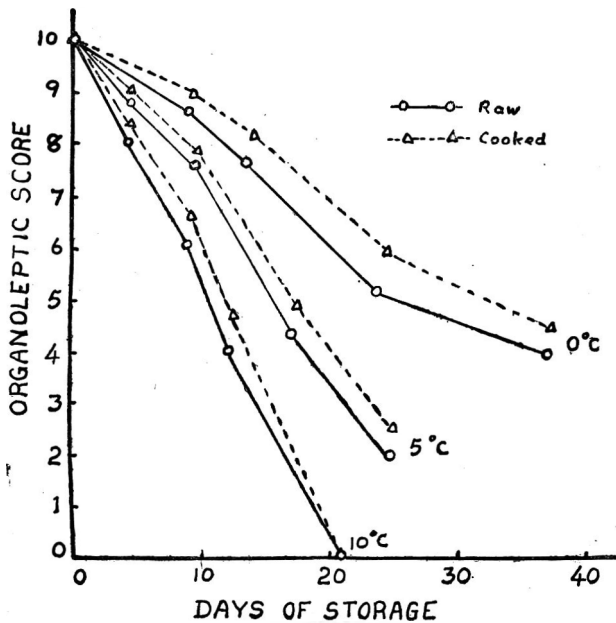


FIG. 1. Changes in organoleptic score of fish stored at different temperatures

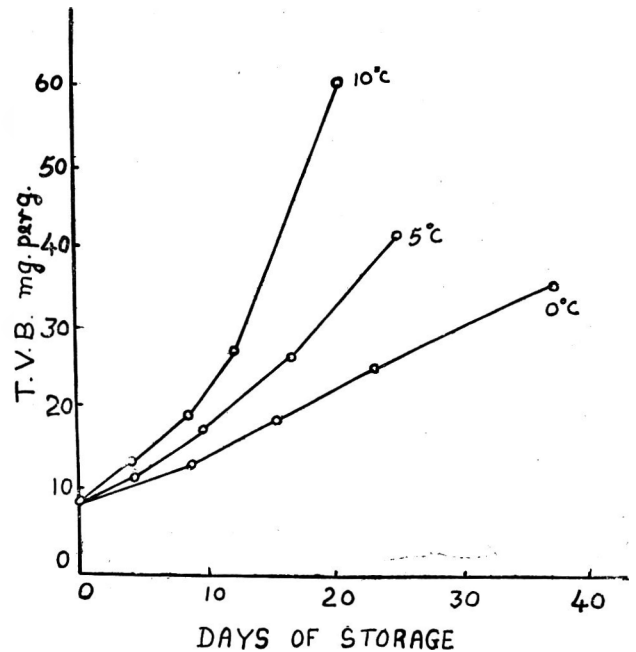


FIG. 2. Changes in total volatile bases in fish stored at different temperatures

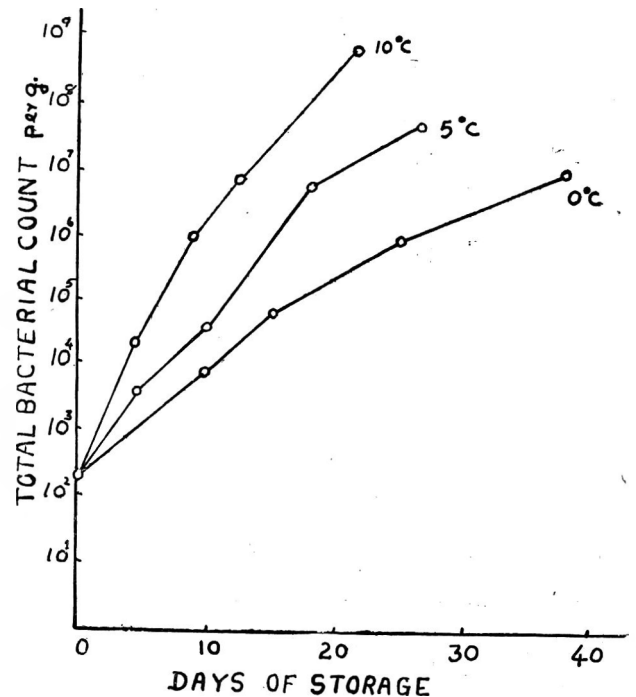


FIG. 3. Changes in total bacterial count in fish stored at different temperatures

Cutting *et al.*<sup>11</sup> for cod. The latter, a typical marine fish spoils at 2.5 times as fast at  $4.4^{\circ}\text{C}$  and 5.5 times as fast at  $10^{\circ}\text{C}$  as they do at  $0^{\circ}\text{C}$ . The rate of acceleration of spoilage with increasing temperature seems to be

TABLE 5. EFFECT OF PHYSICAL DAMAGE OF FLESH ON RATE OF SPOILAGE

Sample	After 7 days in ice		After 15 days in ice	
	TVB mg %	TBC/g	TVB mg %	TBC/g
Sound fish	8.1	$6.7 \times 10^8$	12.3	$7.1 \times 10^8$
Bruised fish	9.5	$1.2 \times 10^8$	22.4	$4.5 \times 10^7$
Tissues from un- affected part of (2) above	8.4	$7.3 \times 10^8$	14.4	$4.4 \times 10^8$
Tissues from bruised part of (2) above	11.3	$1.9 \times 10^8$	29.7	$7.6 \times 10^7$

much lower in the fresh water species under study. The close parallel that exists between spoilage rate of marine fish and the growth pattern of marine psychrophiles responsible for spoilage in respect of temperature changes, has been already established.<sup>7</sup> The present data hence supports the earlier suggestion<sup>1</sup> that spoilage organisms associated with tropical inland sources are very likely different from those found in marine environments.

Results recorded in Table 5 indicate the importance of careful physical handling of fish during catching, transportation and distribution. Even under ideal conditions of storage temperature, bruises occurring in fish accelerates spoilage rate, probably because the natural barriers of skin being damaged, bacterial penetration becomes faster. This is clear from the fact that even in a damaged fish, there is more accelerated spoilage in the bruised parts.

From the results presented here and in the earlier communication,<sup>1</sup> it is evident that the technological problems of handling and transportation of Indian fresh water fish are less formidable as compared to those associated with sea fishes. Being non-fatty, rancidity development may be excluded during the relatively short period of chilled storage. Species

and size differences having been found to exert least influence on spoilage rate, it may be possible to evolve uniform codes for packaging and transport for the major commercial species. Several such natural advantages notwithstanding, a sizeable portion (25-50 per cent) of fish marketed at present go sub-standard.<sup>2</sup> The causes for this can be traced to the inadequacy of packaging and refrigeration, rough handling, and above all, the inordinate delay that occurs before icing. Though icing is at present the best medium of chilling fish, a time may come when this can be conveniently and economically replaced by refrigerated transport. To make the best use of such a system, standardization of factors like ambient humidity has to be worked out. The results also point to the necessity for a detailed study of the microflora associated with fresh water fishes.

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# Essential Amino Acid Content of Goat Meat in Comparison with Other Meats

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**Essential amino acid composition of goat meat as compared with beef, pork and sheep meat (mutton) is presented. Goat meat contains more of arginine, leucine and isoleucine as compared to sheep meat. Pork meat contains more histidine, lysine, methionine, threonine and valine as compared to goat meat.**

In many advanced countries of the world, one quarter of the protein requirements of the population is provided by meat products.<sup>1</sup> Protein from veal, lamb, beef and pork contain liberal amounts of essential amino acids in similar proportion<sup>2-4</sup>. Meat proteins are completely digestible<sup>5</sup> and the biological value is found to be high. Among meat eating sections of the population, goat meat is a very popular article of diet especially in Bihar<sup>6</sup>. At present, the goat population in India is 60 millions. Very little information is available on the amino acid composition

of goat meat, except for a report on the biological value<sup>6</sup>. Work therefore was undertaken to determine the essential amino acid composition of goat meat in comparison with mutton, beef and pork.

## Materials and Methods

Meat samples for analysis were collected from same portions of different animals. Samples from six animals of each species were collected and pooled and one representative sample from each species was analysed. Suitable quantity of meat (goat,

TABLE 1. AMINO ACID COMPOSITION OF MEAT SAMPLES (g/16 g N)

Amino acid	Sheep meat	Goat meat	Beef	Pork	Hen's egg†	FAO Ref. protein‡	Ideal protein§
Arginine*	6.6	7.4	6.8	7.1	...	...	6.6
Histidine*	2.8	2.1	3.0	3.4	...	...	2.4
Lysine	7.9	7.5	8.1	8.7	6.4	4.2	7.5
Tryptophan	1.4	1.5	1.4	1.3	1.6	1.4	1.6
Phenylalanine	3.3	3.5	3.4	3.6	5.8	2.8	5.8
Methionine	3.1	2.7	2.9	3.4	3.1	2.2	2.8
Threonine	4.6	4.8	4.5	5.2	5.1	2.8	5.0
Leucine	7.6	8.4	7.5	8.2	8.8	4.8	10.0
Isoleucine	4.6	5.1	4.5	5.4	6.6	4.2	6.6
Valine	5.5	5.4	4.9	6.0	7.3	4.2	7.0
Tyrosine	3.0	3.1	3.4	3.5	4.2	...	...
Cystine	1.3	1.2	1.1	1.1	2.4	...	...

\* Not included in calculating chemical score

† Reference No. 8.

‡ Literature values

§ Reference No. 9.

TABLE 2. CHEMICAL SCORES OF INDIVIDUAL AMINO ACIDS IN MEAT PROTEINS

Amino acid	Sheep meat	Goat meat	Beef	Pork
Lysine	149	142	156	147
Tryptophan	104	114	108	90
SAA (Me+Cy.)	84	84	89	90
Threonine	87	114	108	113
Phenylalanine+Tyrosine	96	96	93	95
Leucine	104	116	105	137
Isoleucine	85	94	83	90
Valine	91	90	98	92
Protein score	84	84	89	90
Sequence of limiting amino acids	SAA Isoleu. Thr.	SAA Valine Isoleu.	SAA Isoleu. Phe Tyro.	SAA Valine Tyro.

SAA: Sulphur amino acids

mutton, beef and pork) was dehydrated and defatted using acetone. After vacuum drying, the extracted and dried material was powdered. Five hundred mg of the powdered material was hydrolysed with 10 ml of 6N HCl in a vacuum sealed tube at 121°C for 18 hr. After cooling the hydrolysate was adjusted to pH 6.8 and filtered. The filtrate was made upto a known volume. Suitable aliquots were taken for amino acid analysis using microbiological assay procedure<sup>7</sup>. *Leuconotoc mesenteroides* P-60, *Lactobacillus arabinosis* 17/5, *Streptococcus faecalis-R* were used as the test organisms. Tryptophan was estimated in the alkaline hydrolysate using *Lactobacillus arabinosis*. The results are presented in Table 1 representing the average of five replicates.

**Chemical score:** Chemical scores of essential amino acids, and protein scores were calculated according to the procedure recommended by FAO<sup>8</sup>.

The chemical score of individual amino acids of different meats are presented in Table 2. The protein score and also sequence of limiting amino acids are also indicated.

## Results and Discussion

Essential amino acid composition of goat meat in comparison with sheep meat (mutton), beef and pork are presented in Table 1. It can be seen that goat meat contains more of arginine, leucine and isoleucine as compared to sheep meat. The pattern of the remaining amino acids is somewhat similar to that of mutton. Pork meat contains more of histidine, lysine, methionine, threonine and valine as compared to beef meat or meat from goat or sheep. Comparison with the essential amino acid pattern of ideal protein<sup>9</sup> shows that goat meat is comparable with this protein in respect of arginine, lysine, tryptophan, methionine and threonine. The essential amino acids, histidine, phenylalanine, leucine, isoleucine and valine are 87.5, 60.4, 82.0 and 81.8 per cent respectively in comparison with that of ideal reference protein taken as 100.

The chemical scores of individual amino acids are presented in Table 2. The results presented show that goat meat is adequate in respect of all the essential amino acids. The protein score for goat meat is 84 as compared to 84 for mutton, 89 for beef and 90 for pork. The sequence of limiting amino acids is sulphur amino acids followed by valine and isoleucine.

Comparing the results with FAO reference protein pattern all the meats contain more than adequate amounts of the essential amino acids.

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# In-pack Processing of Stuffed *Parottas* in Indigenous Flexible Packaging Materials. Part II. Studies on Alternative Packaging and Processing Systems

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**Two alternate packaging systems were studied and were found unsuitable because of thermosetting of polythene in one case making vacuum packaging difficult and poor flexural resistance of high density polythene in the other. Studies on alternate processing systems indicated that heat damage to the pouches and consequently the spoilage of *parottas* could be reduced considerably (2 per cent only) but not eliminated altogether.**

Storage studies under static condition of a large batch of stuffed *parotta* packs, made by the method of Kannur *et al.*<sup>1</sup> using the indigenous foil laminate, showed that there is a possibility of about 10-12 per cent spoilage due to the failure of the packaging material<sup>2</sup>. These clearly pointed to two possible approaches, if the indigenous packaging materials only were to be utilised viz., (a) alternative packaging systems which could be built up either to make the packaging somewhat more heat resistant or to provide built-in safety in the packs to take care of any post processing contamination arising out of chance failure of the package; and (b) to achieve the optimum processing of the packs under a milder processing system so as to either avoid completely any heat damage to the packaging materials or to bring it down to the minimum, thus contributing to the ultimate success of the packs on long storage. This paper reports results of the experimental work carried out along these lines. An essential feature of the work reported is heat penetration studies and process evaluation for ascertaining the optimum conditions of processing.

## Materials and Methods

**Stuffed *parotta*:** In the different systems studied, each pack consisted of two stuffed *parottas* of same description as reported earlier<sup>2</sup>.

**Special autoclave:** The special autoclave used was as described by Kannur *et al.*<sup>1</sup>, being a large capacity horizontal autoclave of rectangular cross-section. For process evaluation at different processing conditions a smaller capacity (3.5 cft) vertical

autoclave having the same essential features as described for the large capacity autoclave was used.

**Packaging systems:** The following systems were studied: (i) Immediate packaging of MST cellophane 300 followed by 60 g kraft/.04 mm foil/150 gauge polythene. (ii) Immediate packaging of MST cellophane 300 followed by wrapping with sorbic acid treated crepe paper (2 g sorbic acid/sq m) final packaging of 60 g kraft/.04 mm foil/150 gauge polythene. (iii) Immediate packaging of MST cellophane 300 followed by 300 gauge high density polythene. (iv) System (i) packed under vacuum. (v) System (ii) packed under vacuum. (vi) System (iii) packed under vacuum.

**Processing systems:** The following processing conditions were used:

(i) 121°C (15 lb steam pressure), 35 min from come up time.

(ii) 121°C with steam and super imposed air pressure to a total retort pressure of 30 lb and time adjusted to bring F° approximately to the same value as applied to packaging system (i).

(iii) 121°C with steam and superimposed air pressure to a total retort pressure of 30 lb after initial steaming at 100°C till the temperature at the core of the *parotta* reached 98°C. The time of heating at 121°C was adjusted till a process value of 3.0 was reached.

(iv) 115°C with steam and superimposed air pressure to a total retort pressure of 20 lb. Initial steaming and subsequent heating at 115°C were carried out as in (iii) above.

(v) 110°C with steam and superimposed air pressure to a total retort pressure of 15 lb. Initial steaming and subsequent heating at 110°C were carried out as in (iii) above.

**Temperature recording system:** The probes for recording the temperature in the packages consisted of copper-constantan thermocouples connected to potentiometer through selector switch. The thermocouple hot junction was placed at the point of maximum heat lag in the package determined experimentally and the exit of the wires through the pouch was sealed with araldite. For efficient sealing in case of foil laminate, the outer paper layer at the point of exit was carefully removed before applying the araldite and in case of high density polythene since the bond is not very strong the wires were taken out through the seam at one corner and the entire area at the seam over the wire on both sides of the pouch was coated with araldite. The cold junctions of the thermocouple were dipped in ice-water. Potentiometric readings were converted into temperature with the help of a conversion chart.

**Determination of the point of maximum heat lag in the pack:** The probes were positioned inside the stuffed *parotta* pack at various points and temperatures were recorded during a heat processing operation (35 min at 121°C). From these observations the point of maximum heat lag was confirmed to be the centre between the two *parottas* as could indeed be expected.

**Process evaluation:** This was done by a simplified procedure avoiding the need of plotting the heat penetration data as in the classical methods. The method followed was that of Patashnik<sup>7</sup> by which temperature readings were taken at equal time intervals during the heat penetration test and the corresponding lethality values (F/t) were tabulated by reference to a table giving lethality ratios for different observed temperatures when the reference temperature is 121°C and Z=18. The product of this sum ( $\Sigma F/t$ ) and the equal time interval gave the process value directly.

**Storage and examination of packs:** The packs after processing were enclosed in groups of five in corrugated board cartons and stored under ambient condition (25-35°C) and examined for microbiological count and sensory evaluation at intervals of 1 month during a storage period of 6 months.

## Results and Discussion

It is known as a result of a great deal of work already done in the field of autoclavable flexible food packs,<sup>4-6</sup> that certain laminates are most suitable.

The essential common feature of these laminates is the heat resistant innermost polymer layer which enables the pouches being used at a processing temperature of 121°C or higher. These types of heat resistant foil laminates, however, are not indigenously produced in this country at present and it was in this context that the initial success of Kannur *et al.*<sup>1,3</sup> in heat processing stuffed *parotta* in kraft/foil-polythene pouches aroused a great deal of interest. The results of a large scale trial, previously described,<sup>2</sup> pointed out to the limitations of these pouches. The present investigations were therefore, undertaken to bring about any possible improvement in the situation by making suitable alterations in the packaging and processing systems.

In the first stage, some alternative packaging systems, as mentioned above, were used and their comparative heat penetration characteristics were studied keeping the processing conditions same as used by Kannur *et al.*<sup>1</sup> viz., 35 min retort heating at 121°C. It was not necessary for the purpose of this study to ascertain first, the suitability or otherwise of this processing condition. Process value (F°), which is an index of amount of heat received by the packs, was determined in each case by the method of Patashnik<sup>7</sup> as this was found to be very convenient for this study. Among the alternative packaging materials tried, high density polythene pouch was chosen because of its higher softening temperature than that of low density polythene and sorbic acid wrapper was used in one system with the hope that it will prevent any possible post processing fungal contamination arising from physical damage to the pouch during handling and transportation. Vacuum packaging was resorted to in some of the systems because of its obvious advantage, in facilitating quicker heat penetration as indeed could be seen from Table 1. Table 1 also shows that in systems where high density polythene pouch and sorbic acid wrapper along with paper-foil-polythene pouch have been used, heat penetration is very poor. This is no doubt due to greater amount of air which get automatically enclosed inside these packs than in case of MST cellophane 300 and paper-foil-polythene pouch. Vacuum packaging could be resorted to as a counter measure provided vacuum was uniformly retained. In the case of paper-foil-polythene pouches this is not so; occasionally the seam opens up during processing causing partial loss in vacuum. This explains why the two packs made of paper-foil-polythene pouches with evacuation did not give the same F° on processing. In the case of the high density polythene pouches though there is no indication of the seams opening up during processing,

the advantages of its higher softening point is lost to some extent due to its poor flexural resistance and its less efficient heat sealing characteristic as a result of which occasionally the packs developed cracks near the seams. The alternative packaging systems studied, therefore, could not be of any practical use.

In the second stage, alternate processing systems were studied with the aim of minimising heat damage

to the pouches either by cutting down the time of heating or lowering the temperature of retort processing or both. Pflug<sup>8</sup> had reported the advantages of air-steam mixture during retort heating and this technique was used to bring down the processing time. The method followed for employing superimposed air pressure for processing stage also was to first build up the air pressure inside the retort to the extent which will be given by steam alone to attain the desired temperature and then to introduce steam till the required temperature is attained and the total pressure also doubles up. It was found that by manipulating the steam and air pressure regulators it was not difficult to maintain the retort pressure and temperature at a constant level. As a result of using this method of processing, the time taken at 121°C to attain approximately  $F^{\circ}=6.5$  is brought down to 24 min from 35 min (Table 2). A further reduction in processing time could be brought about by lowering the  $F^{\circ}$  value. The processing followed by Kannur *et al.* which gave a  $F^{\circ}$  value of 6.4 was obviously excessive and was adopted as an extreme measure of precaution to eliminate any chance of underprocessing in any of the packs. If the lethality values are calculated with respect to one of the most heat resistant and toxin producing bacteria i.e. *Clostridium botulinum* as is usual with canned food processing, then a  $F^{\circ}$  value of 2.78 is adequate as was shown by Esty and Meyer<sup>9</sup>. This value was rounded off to 3 for our experiments to allow for variations that are possible in a large batch due to differences

TABLE 1. PROCESSING OF STUFFED PAROTTA IN DIFFERENT PACKAGING SYSTEMS

Packaging systems	Process value	Sterility
MST cellophane 300, paper-foil-polythene pouch, normal packaging.	6.44; 6.41	Sterile
„ vacuum packed.	17.68; 7.56*	Sterile
MST cellophane 300, 300 gauge high density polythene pouch, normal packaging.	0.64; 0.01	Not sterile
„ vacuum packed	11.51; 12.26	Sterile
MST cellophane 300, wrapping with sorbic acid paper, packaging in paperfoil-polythene pouch, normal packaging.	1.15; 1.51	Not sterile

\* Vacuum was not retained in the pack showing lower process value.

Retort operation at 121°C for 35 min.

TABLE 2. PROCESSING OF STUFFED PAROTTA PACKS UNDER MILDER PROCESSING CONDITIONS

Processing method	Retort temp °C	Retort pressure (lb/sq. in.)	Processing time (min.)	$F^{\circ}$ value	After storage for 6 months under ambient conditions			
					Contaminated packs* (Nos.)	Organoleptic rating†	Pouches with seam gap‡	Pouches with pin holes/cracks‡
Steam alone	121	15	35	6.4	10 out of 100	Acceptable	1 in 4	1 in 2
Super imposed air pressure	121	30	24	6.8	...	...	...	...
Initially steamed (98°C) and processed with super imposed air pressure	121	30	12	3.2	2 out of 96	Acceptable	-ve in all 20	1 in 2
„	115	20	26	3.6	Nil	„	„	„
„	110	15	52	3.3	2 out of 96	„	„	„

\* About 16 nos of packs were opened every month and tested for microbiological counts after incubation both at 37°C for 7 days and 55°C for 7 days. A total plate count of more than 10 per g was considered as a +ve test for contamination. The total number of cases of +ve test thus found at the end of 6 months was reported.

† Organoleptic evaluation was carried out every month by a panel of 5 judges, the ratings being 'as good as fresh', 'acceptable and not acceptable'.

‡ These tests were carried out as described by Ghosh *et al.*<sup>2</sup>

in the thickness of the *parottas* and also in the amount of enclosed air which could not be avoided. Examination of a large number of packs subjected to retort processing under conditions standardised to give this value has confirmed that the packs are all rendered microbiologically sterile. Table 2 shows that with  $F^\circ=3$  as the target, the heat processing of the packs could be completed in 12 min. Thus the processing time could be cut down to nearly 1/3 the time taken previously by Kannur *et al*<sup>1</sup>. In the two other processing systems studied, lower temperatures of 115°C and 110°C were used with the corresponding amount of superimposed air pressures. The processing time in these cases are found to be 26 and 52 min respectively (Table 2, serial nos 4 and 5). The effect on the pouches of processing under the above three milder conditions, viz., 121°C for 12 min, 115°C for 26 min, and 110° for 52 min to achieve the same process value, was examined qualitatively as previously done<sup>2</sup>. It was observed that though the white patch formation was still there in all cases, the pouch defects i.e. gaps in the seam and pinhole/crack across the pouch body was less than what was found previously (Table 2). Storage studies of large batches of packs processed under these three processing conditions were carried out to find out if the apparently better behaviour of the pouches is reflected in the reduction of spoilage due to microbiological attack. The results as noted in Table 2, clearly indicates the

marked improvement. It could be concluded therefore, that by reducing the process value from  $F^\circ=6.4$  to  $F^\circ=3$  and also by using superimposed air pressure during the processing stage which resulted in quicker heat penetration the heat damage to the pouches could be considerably reduced and consequently the chances of spoilage could also be brought down to about 2 per cent. With low density polythene based foil laminate as the pouch material it appears that this is the limit of improvement which can be effected.

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## Studies on the Dehydration of *Dahi* (Milk Curd)

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***Dahi* has been dried by various methods and the quality evaluated. Spray drying and infra-red drying gave products better than the one from tray drying process. However, freeze dried product is better than even these. A new drying technique known as air-diffusion (dispersion) drying has been tried which gave a product similar to freeze dried one and its reconstitution properties were found even better. The detailed study with the air diffusion drying process has been reported in the paper.**

*Dahi* is one of the most popular fermented milk products consumed in India. The production of milk in India is estimated to be about 23.0 million tons<sup>1</sup> of which 9.1 per cent is utilised for making curd. In India curd is generally prepared by inoculating mixed cultures of lactic acid organisms and the final curd quality is dependent upon the type of the ino-

culating organisms<sup>2,3</sup>. In curd, not only the quality of milk is retained but also its digestibility is improved<sup>4</sup>.

Though the storage life of curd is much more than that of milk, yet it is a perishable commodity and is rendered unsuitable for human consumption fairly quickly. If *dahi* can be preserved for use over



longer periods by dehydration it will be a popular ready-to-eat food.

Very little information is available on the dehydration of *dahi*, except a report by Bhatia *et al.*<sup>5</sup>. None of the usual drying methods are found to be successful in giving a product which has the same curd tension after reconstitution. The present work was, therefore, undertaken to develop a suitable method of drying *dahi* which on reconstitution gives a product similar to fresh *dahi*.

### Materials and Methods

**Preparation of dahi:** Milk was heated in a water bath at 85°C for 30 min with frequent shaking of the contents of the flask and cooled immediately to room temperature. This was then inoculated with *S. thermophilus* grown on sterile milk (5 psig, 30 min) at the rate of 5 per cent (v/v) and incubated for 16 hr at 40°C. The titratable acidity of the inoculum was between 0.80 and 0.85 per cent lactic acid.

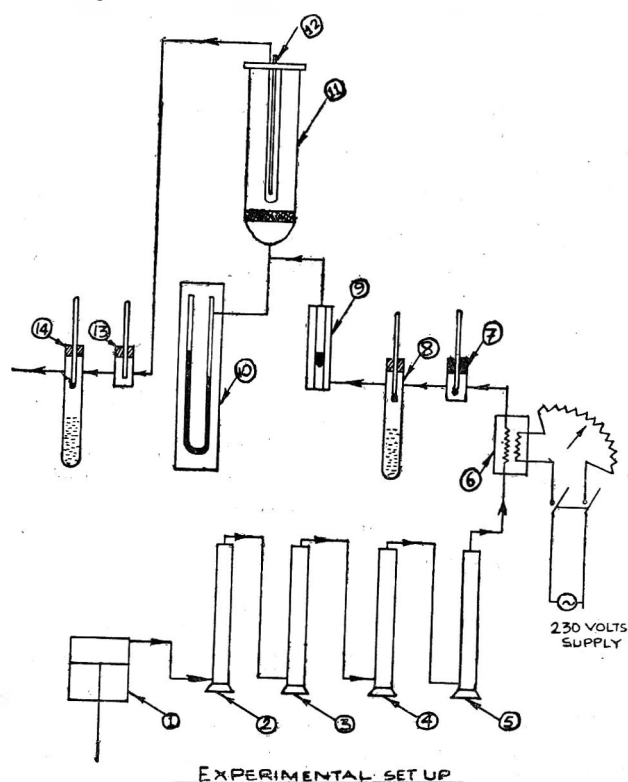
**Drying methods:** The drying techniques applied were atmospheric tray, vacuum tray, spray, infra-red and freeze dryings. The atmospheric tray dryer was an electrically heated cabinet type through flow dryer. The vacuum tray dryer was also electrically heated and fitted with a high vacuum pump. In the infra-red drying unit air was blowing in the cabinet within which material was placed for drying under an infra-red lamp. The voltage and the time of operation could be controlled. Also the weight loss of material due to evaporation of moisture during drying could be recorded by a suitable arrangement. Laboratory models of these drying units were used.

**Method of analysis:** Titratable acidity and total bacterial count were determined according to the method of Indian Standards Institution<sup>6</sup>. Volatile acidity was determined according to Hammer and Bailey<sup>7</sup> and expressed as ml of N/100 NaOH required to neutralise 150 ml condensate collected from 2.5 g dried powder.

**Reconstitution property:** The reconstitution property was measured organoleptically. Five grams dry powder was taken in a beaker and reconstituted with warm water (60°C) upto 20 per cent total solid concentration and allowed to stand for 5 min. The product was then tested organoleptically for colour, flavour, homogeneity and taste by a panel of five persons. The members of the panel were selected previously by their capacity to judge the product. Each member was asked to give marks on the above factors out of 10 assigned to them and then an average was taken. When the average comes to 7-9 the

product was said to be good, 5-6.5 fair and 4 and below as poor.

**Experimental set up for air-diffusion drying:** This was installed using dehumidified air diffusing through a sintered glass disc over which curd was placed (Fig. 1). When dehumidified air was passing through the material, moisture from the curd took its latent heat of vaporisation from the material itself with subsequent cooling and transferred to the vapour phase due to the difference in partial pressure of water vapour in incoming air and in the curd itself. So by using air at a temperature of 25°C and RH 10 per cent, drying was virtually taking place at about 15°C. The air was dehumidified by using a number of silica gel towers. The inlet and outlet air conditions were determined from the records of wet and dry bulb thermometers. The air flow rates were measured with the help of a rotameter provided across the line and pressure drop at the sintered glass disc by a Hg-manometer.



- EXPERIMENTAL SET UP
1. Compressor
  - 2, 3, 4, 5, Dehumidifier (silica gel tower)
  6. Heater with regulator
  - 7, 13, Dry bulb thermometer
  - 8, 14, Wet bulb thermometer
  9. Rotameter
  10. Mercury Manometer
  11. Sintered glass tower
  12. Thermometer pocket

FIG. 1. Schematic diagram showing the various units of the experimental set up for air diffusion drying of curd

Drying experiments were done by using various sintered glass diffusers, different flow rates of air and material bed heights. The air pressure was kept constant at 30-40 psig. The inlet air humidity ( $10 \pm 0.5$  per cent) and temperature ( $25 \pm 1^\circ\text{C}$ ) were also kept constant.

### Results and Discussion

The results of various drying operations tried under different conditions as well as the quality tests carried out are given in Table 1. The analysis of various products obtained by air-diffusion or dispersion drying under various drying conditions are given in Table 2.

Of all drying processes employed freeze drying and air-diffusion drying gave the best results. Air diffusion drying gave a product close to the quality of freeze dried product with even better reconstitution properties. It was seen that the curd, if dried at more

than  $30^\circ\text{C}$ , the reconstitution property was very poor. For this reason the other drying methods such as atmospheric and vacuum tray, spray and infra-red drying were found to be not at all suitable for drying of curd. Spray drying, however, found to be better than tray drying operations because of less time of contact with the drying medium (hot air). Again of all tray drying processes infra-red drying rate was very fast because of the penetration of high energy IR-rays, and quality of product was also better than those obtained from atmospheric and vacuum tray drying processes.

In air-diffusion drying the temperature of the material (*dahi*) gradually fell from room temperature upto  $14-15^\circ\text{C}$  under the conditions of the experiment and remained constant at constant drying rate period and then gradually rose upto  $20^\circ\text{C}$  during falling rate period. This was because the rate of moisture evaporation was very high at constant drying rate

TABLE 1. DRYING TRIALS AND QUALITY EVALUATION OF DRIED DAHI POWDER

Drying methods	Drying conditions	Final moisture %	Bacterial count/g	Total titratable acid (% lactic)	Volatile acid (ml of N/100 NaOH)	Reconstitution	Colour and flavour
Atmospheric tray	Drying temp= $55^\circ\text{C}$ Air rate= $7.1\text{m}^3/\text{min}$	5.4	$7 \times 10^4$	5.62	4.25	Very poor	Turns brown and charred milk flavour
Vacuum tray	Drying temp= $45-50^\circ\text{C}$ Vacuum= $63.5\text{ cm Hg}$	3.6	$5 \times 10^4$	5.11	4.38	Poor	„
Spray drying	Drying temp= $178-180^\circ\text{C}$ Material flow= $50\text{ ml/min}$ Air pressure= $40-50\text{ psig}$	7.5	$2 \times 10^8$	6.78	6.25	Highly loose texture	Slightly brown and poor flavour
Infra-red	Drying temp= $30-35^\circ\text{C}$ Voltage= $250\text{ Volts}$ Air rate= $6.5\text{ m}^3/\text{min}$	5.5	$3 \times 10^4$	5.06	6.86	„	„
Freeze drying	Working pressure= $0.02\text{ cm Hg}$	3.4	$20 \times 10^7$	5.05	8.12	Good texture	Excellent colour and flavour

TABLE 2. DIFFUSION DRYING OF DAHI UNDER VARIOUS DRYING CONDITIONS

Diffusion dried sample No.	Drying conditions				Final moisture (%)	Bacterial count/g	Total titratable acid (% lactic)	Volatile acid (ml. N/100 NaOH)
	Air flow rate (cc/min)	Diffuser size (micron)	Dahi bed ht (cm)	Wt. of dahi (g)				
R-1	6,000	20-30	2	200	4.1	$85 \times 10^6$	4.27	5.06
R-2	6,000	20-30	3	290	4.3	$101 \times 10^6$	5.11	7.76
R-3	8,000	20-30	2	205	5.4	$205 \times 10^6$	4.48	8.22
R-4	6,000	5-15	3	300	5.3	$130 \times 10^6$	5.53	8.41
R-5	8,000	5-15	3	300	4.1	$165 \times 10^6$	5.12	9.31
R-6	8,000	5-15	2	200	5.8	$98 \times 10^6$	5.16	8.86
R-7	6,000	40-50	2	205	5.5	$109 \times 10^6$	5.35	6.86
R-8	8,000	40-50	2	205	5.6	$126 \times 10^6$	4.74	6.70
R-9	6,000	100-120	2	200	5.3	$121 \times 10^6$	4.81	6.16
R-10	8,000	100-120	2	200	5.0	$78 \times 10^6$	5.35	5.31

All possessed excellent texture and flavour but slightly poorer colour than freeze dried product.

period but not so at the falling rate period<sup>8-9</sup>. When equilibrium was reached the temperature of the material and that of the incoming air was same as there was no further evaporation of moisture.

Under the conditions of the experiments the sintered glass diffuser of 20-30 micron and air flow rate 8000 cc/min were found to be optimum. The special advantage of air diffusion drying was that the product quality was good, drying rate was faster and much cheaper than freeze drying process.

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## Changes in Ascorbic Acid and Carotene Content of Green Leafy Vegetables on Cooking

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**The effect of cooking by frypan, under pressure and by the traditional method on ascorbic acid and carotene in amaranthus, bathua, beetroot leaves, cabbage, fenugreek, knol khol leaves, radish leaves and spinach has been studied. The losses are minimum in pressure cooking and maximum in traditional method.**

Green leafy vegetables are a good source of ascorbic acid, carotene and B-complex vitamins and minerals like iron, calcium and phosphorus.

Data available on the losses of nutrients that occur when vegetables are cooked by household methods in India are meagre. Therefore, an attempt has been made, in this study to investigate the losses of ascorbic acid and carotene in green leafy vegetables during cooking.

### Materials and Methods

*Raw material:* Amaranthus (*Amaranthus viridus*), bathua (*Chenopodium album*), beetroot leaves (*Beta vulgaris*), cabbage (*Brassica oleracea* var. *capitata*), fenugreek leaves (*Trigonella foenum graecum*), knol khol leaves (*Brassica oleracea* var. *cauloporapa*), radish

leaves (*Raphanus sativus*), and spinach (*Spinacia oleracea*) were taken for the present study.

*Preparation:* Fresh leaves free of stalks or damage were thoroughly washed in a colander with tap water drained and chopped into about  $\frac{1}{4}$  in wide strips with a stainless steel knife.

*Cooking:* Three methods of cooking as described below were followed. The time required for cooking and the weight of the cooked samples were recorded.

*Fry-pan cooking:* The required quantity of water (Table 1) was brought to a boil in an aluminium fry-pan, 100 g of the prepared vegetable added, covered with the lid and cooked on a low flame, till the vegetable attained required degree of softness and there was no left over water.

TABLE 1. COOKING CONDITIONS

Vegetables	Method of cooking	Amount of water (ml)	Cooking time (min)	Cooked wt. (g)
Amaranthus	a	170	8	92
	b	85	1	102
	c	200	11	90
Bathua	a	170	9	90
	b	85	1	100
	c	200	16	88
Beetroot leaves	a	170	8	96
	b	85	2	94
	c	220	14	90
Cabbage	a	60	15	95
	b	85	2	92
	c	60	21	85
Fenugreek	a	85	15	95
	b	85	1	95
	c	100	19	90
Knol-khol leaves	a	250	23	95
	b	85	8	95
	c	270	28	80
Radish leaves	a	390	20	98
	b	85	4.3	92
	c	450	28	85
Spinach	a	...	6	85
	b	85	1	95
	c	15	9	70

a, Fry-pan cooking; b, Pressure cooking;  
c, Traditional method

Starting material, 100g of vegetable

**Pressure cooking:** 100 g of the prepared vegetable was added to 85 ml of boiling water, covered and cooked in a pressure cooker until the pressure raised to 15 psig.

**Traditional cooking:** 100 g of sample was placed in the requisite quantity of water in a saucepan and

cooked on a low flame till there was no left over water. The time required for cooking was noted.

**Analysis:** The samples were homogenized in a blender. Two grams of the sample were taken in triplicate for analysis of ascorbic acid and carotene. Ascorbic acid was determined by the method of Roe and Kuether as described by Caraway<sup>1</sup> and carotene by the method of Davis as modified by Beerh and Siddappa.<sup>2</sup>

Analysis of variance was done to test the significance of the differences in mean values between different methods of cooking.

## Results and Discussion

**Ascorbic acid:** Ascorbic acid content in fresh leafy vegetables varied from 20 mg in spinach to 123 mg/100 g in knol khol leaves (Table 2). Gopalan *et al*<sup>3</sup> have reported slightly higher values. The percentage retention of ascorbic acid varied from 32.2 to 61.2 in the fry-pan method, 45.9 to 80 in pressure cooking and 44 to 28.5 in traditional method of cooking. The losses were least in pressure cooked samples and the highest in vegetables cooked by the traditional method; the differences were significant ( $P < 0.01$ ). The losses of ascorbic acid in cabbage were 20.8, 45.1 and 59.5 per cent by pressure, fry-pan and traditional methods of cooking respectively. The values reported in literature on the per cent losses vary from 26-31, 59-60, 45-36 and 22-35 for pressure cooked, boiled, cooked in tightly covered pan and steamed cabbage respectively.<sup>4-6</sup> Spinach showed a loss of 28.5, 72.9, 32.5 and 32.7 per cent when cooked similarly. Gordon *et al*<sup>7</sup> have reported 22 per cent loss in pressure cooking, 37 per cent in tightly covered pan and 36 per cent

TABLE 2. AVERAGE ASCORBIC ACID AND CAROTENE CONTENTS OF RAW AND COOKED VEGETABLES

Vegetables	Ascorbic acid						Carotene					
	Raw (mg/100g)	Cooked			F value*	C.D.	Raw ( $\mu$ g/100g)	Cooked			F value*	C.D.
		a	b	c				a	b	c		
Amaranthus	93.33	30.09	42.83	26.62	5.4	36.35	5183	4417	5033	4353	1244.0	87.47
Bathua	47.35	18.93	23.49	17.60	64.2	0.23	3097	2619	2988	2498	10.5	264.9
Beetroot leaves	67.91	26.64	34.02	21.92	2175.0	0.57	5266	4848	4792	4460	1689.3	16.38
Cabbage	112.50	61.75	89.05	45.52	6412.0	1.45	1229	1088	1092	932	157.5	37.94
Fenugreek	52.91	27.48	32.65	23.05	1040.0	1.51	2458	2189	2207	2073	1400.1	50.55
Knol khol leaves	123.25	48.48	57.38	41.24	1150.4	34.31	4251	3864	3992	3542	7115.0	0.568
Radish leaves	85.63	49.52	60.56	32.16	3701.5	1.45	4981	4602	4359	3948	1967.1	39.2
Spinach	20.30	12.41	14.74	7.10	450.0	0.96	5222	4394	4937	3569	4896.4	41.5

\* Significant at 1% level. a, Fry-pan cooking; b, Pressure cooking; c, Traditional method of cooking.

in steamed spinach. The losses in fenugreek on cooking by these methods were 38.3, 48.1 and 56.4 per cent. Rowland *et al.*<sup>8</sup> have reported lower losses of ascorbic acid on cooking fenugreek by boiling, steaming and frying. Of all the vegetables studied amaranthus showed the highest loss i.e. 54.1, 67.8 and 71.5 per cent by pressure, fry-pan and traditional cooking respectively.

The percentage losses for other vegetables were 50.4, 60.1 and 62.8 in *bathua*, 49.9, 60.8 and 67.7 in beetroot leaves, 53.4, 60.7 and 66.5 in knol khol leaves, 29.2, 42.1 and 62.4 in spinach by pressure, fry-pan and traditional method of cooking respectively.

**Carotene:** The carotene content of raw vegetables (Table 2) ranged from 1229  $\mu\text{g}$  in cabbage to 5266  $\mu\text{g}/100\text{ g}$  in beetroot leaves. Similar values have been reported by Gopalan *et al.*<sup>3</sup> The losses were the highest in cooking done by the traditional method and the least in the pressure cooked vegetables. The losses by the three methods of cooking are significant ( $P < 0.01$ ). The losses in spinach were 5.5, 15.8 and 24.2 per cent by pressure, fry-pan and the traditional method of cooking respectively. Losses reported vary from 15-17 per cent. The percentage losses were 2.9, 14.7 and 16.0 in amaranthus, 3.5, 15.4 and 19.3 in *bathua*; 9.0, 7.9 and 15.3 in beetroot leaves; 11.0, 11.4 and 24.2 in cabbage; 10.2, 10.9 and 15.7 in fenugreek; 8.4, 9.1 and 16.6 in knol khol leaves; 12.4, 7.6 and 20.7 in radish leaves by pressure, fry-pan and traditional method of cooking respectively. Steenback and Bontwall,<sup>10</sup> Booher *et al.*<sup>11</sup> and Peter *et al.*<sup>12</sup> have, however, reported an increase in carotene content of cooked vegetables.

On the whole, losses of ascorbic acid were more than those of carotene and were the highest in vegetables cooked by the traditional method and the least in pressure cooked vegetables. Higher losses in the traditional method of cooking may be due to (i) the enzyme oxidation during the time lag between preparation and cooking<sup>13</sup>, (ii) longer time required for

cooking, and (iii) the practice of cooking without the lid accompanied by frequent stirring which exposes the material to atmospheric oxidation. Lower losses of ascorbic acid during cooking in the fry-pan and pressure cooker might be partly due to the destruction of the enzyme when the prepared material was placed in boiling water. Boiling of water eliminates dissolved oxygen and cooking in closed container excludes atmospheric oxygen.

The results of the present investigation clearly indicate that pressure cooking helps in the retention of ascorbic acid and carotene of leafy vegetables. The losses of these nutrients in the traditional method of cooking may be reduced by placing the chopped vegetables in minimum quantity of boiling water and cooking in tightly covered pan without stirring.

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## RESEARCH NOTES

### VARIETAL DIFFERENCES IN PUFFING QUALITY OF PADDY

Wide varietal variation has been found in the puffing quality of paddy. There seems to be no consistent relation between puffing quality and amylose content, although some waxy types were the best puffers. There was no relation between protein content and puffing quality.

Varietal differences in puffing quality of paddy are known in rice producing countries. Mottern *et al.*<sup>1</sup> reported such differences for U. S. varieties. Recently puffing quality studies for about 40 Indian varieties of paddy were carried out by Lakshmi and Desikachar<sup>2</sup>. Since completion of this work in this laboratory, it has been shown that sun checks produced in the grains by late harvesting or severe drying adversely affect the puffing quality of paddy<sup>3</sup>. In an investigation to breed paddy strains with best puffing

quality, it was necessary to screen a large number of varieties for their basic puffing potential uninfluenced by adverse harvest and drying practices. With this in view, pure bred varieties grown in the Regional Research Station, Mandya, were harvested at moisture level between 20-24 per cent, dried in the shade to 14 per cent moisture and used for the puffing studies. The formation of sun checks caused by late harvesting and over drying was thus minimised.

Puffing quality was determined as per details described earlier<sup>3</sup>. Protein was determined by Microkjeldahl method<sup>4</sup> while amylose estimation was carried out as per the method of Sowbhagya and Bhattacharya<sup>5</sup>. Percentage of sun checked grains was determined using a crack detector unit developed in this laboratory<sup>6</sup>.

Relevant data on the various varieties are presented in Table 1. The varieties arranged in descending

TABLE 1. VARIETAL DIFFERENCES IN PUFFING QUALITY OF PADDY

Sl. No.	Variety	Puffing yield per 100g of paddy		Vol. index†	Sun checked grains%	Amylose%	Protein%
		Vol. (ml)	Wt (g)				
1	S-2222	1160	58.8	19.7	2	22.5	7.5
2	Y-4	1130	59.5	19.0	0	19.9	7.7
*3	Korean Waxy	1040	60.0	17.3	N.D.	3.4	10.6
4	S-317	1000	54.2	18.4	0	N.D.	N.D.
5	S-701	1000	53.0	18.9	6	20.9	7.9
†6	IR-253	920	60.0	15.3	N.D.	3.4	9.2
†7	White Puttu	920	54.2	17.0	N.D.	8.6	8.3
8	S-749	850	49.0	17.3	1	20.0	8.5
9	Jenugudu	800	43.5	18.4	31	N.D.	N.D.
10	BAM-3	790	47.5	16.6	16	20.9	7.1
11	C-435	760	45.9	16.5	35	17.1	7.5
12	T-65	720	47.2	15.2	40	15.7	7.2
13	GEB-24	688	45.9	15.0	N.D.	20.6	7.5
14	IR-20	680	48.7	13.9	39	N.D.	N.D.
15	B-1370	672	51.2	13.1	N.D.	21.0	7.7
16	Taiwan	640	44.1	14.5	N.D.	16.3	7.5
17	Madhu (MR 136)	640	41.0	15.6	57	N.D.	N.D.
18	S-1092	600	34.7	17.2	41	21.7	7.1
19	Chippiga	592	40.0	14.8	N.D.	21.5	7.7
20	IET-1991	560	38.9	14.4	34	N.D.	N.D.
21	S-199	540	39.6	13.6	19	21.1	8.8
22	T-141	480	34.2	14.0	32	20.8	7.2
†23	CO-10	360	30.2	11.9	36	21.9	9.4
†24	Purple Puttu	360	35.2	10.2	N.D.	3.0	15.0
25	P.T.B-10	320	31.6	10.1	20	21.9	9.5
26	Ch-2	300	30.4	9.8	26	20.2	6.7
27	IR-5	160	15.2	10.5	47	19.6	N.D.
28	Mgl-1	140	16.0	8.7	12	21.1	7.9
29	Pankaj	140	14.0	10.0	35	17.6	9.5
30	IR-8	7	0.8	8.7	30	19.0	7.1

\* A waxy rice procured from South Korea and stored for 2 years.

† Procured from Agricultural Research Institute, Coimbatore.

‡ Ratio of bulk volume to weight of puffed material.

N.D.: Not done

order of the puffing volume reveal wide differences with regard to the puffing quality of paddy. The data indicate that the first 6 or 7 varieties of paddy have a good potential with regard to high puffing quality both in terms of weight as well as the bulk volume of the puffed grains. About 10 other varieties could be considered as medium puffers while the rest should be considered as having poor puffing quality.

There does not seem to be any relation either between the protein content and the puffing quality of the grain, or between amylose and puffing quality although three of the best puffers ('Korean Waxy', 'IR-253' and 'White Puttu') have a very low proportion of amylose. As the per acre yield of these waxy varieties is low, they do not seem to have potential for development. The varieties 'S-2222', 'Y-4', 'S-317' and 'S-701' have fairly high puffing quality both in terms of yield and bulk volume of puffed product. Some of these are widely cultivated for their superior agronomic and grain qualities. The per acre yield is also fairly high. It is interesting to note that in those varieties which had good puffing quality the percentage of sun checked grains was also surprisingly low. These could, therefore, form parent material for genetic improvement of varieties with maximum puffing volume and with minimum proportion of non-puffed grains. There have been indications in preliminary studies that chalkiness as well as abdominal white character of grains contribute to low puffing quality<sup>7</sup>.

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## EFFECT OF HOMOGENIZATION AND PASTEURIZATION ON RELATIVE VISCOSITY OF MILK

The sequence practised was homogenization prior to pasteurization (HPP) and homogenization after pasteurization (HAP). The average relative viscosity of raw buffalo milk was 1.8695 which increased by 3.5 and 1.6 per cent on pasteurization by holder and high heating temperature method. Increases in relative viscosity amounted to 86.2 per cent and 57 per cent over raw milk for HAP holder single and double stage pasteurised milk respectively, in comparison to 63.4 per cent and 46.4 per cent respectively in case of HPP holder for similarly treated milk. For HAP high temperature heating, single and double stage pasteurized milk resulted in increase in relative viscosity to 80.9 and 55.8 per cent respectively in comparison to 61.7 and 44.9 per cent respectively in case HPP high temperature heating for similarly treated milk. The above results indicated that single stage homogenisation is more effective than double stage and HAP is more effective than HPP method for increasing the viscosity.

The richness of milk is commonly judged by the consumers mostly in terms of its viscosity. The processing techniques adopted in dairy industry too, depend on the viscosity of milk. Milk fat is one of the constituents affecting milk viscosity. Amongst the mechanical means adopted, homogenization is known to increase the milk viscosity. Evenson and Ferris<sup>1</sup> observed a considerable increase in viscosity of natural cow milk pasteurized at 63°C and homogenized at 43.3°C and that the higher the homogenizing pressure, higher was the viscosity. Halloran and Trout,<sup>2</sup> Halloran and Gould<sup>3</sup> noted decreased viscosity on pasteurization and homogenization of cow milk. Doan<sup>4</sup> recorded increased viscosity on homogenization and stated that this increase in viscosity was not great unless the fat content was increased to 5 or 6 per cent. Kulkarni and Dole<sup>5</sup> reported average viscosity of Delhi breed buffalo milk of 1.355 at 30°C. Puri and Gupta<sup>6</sup> noticed that storage of milk for 10 hr at 15-25°C, for 6 hr at 30°C and 3 hr at 35°C did not have noticeable change in its viscosity.

In the present investigation, the effect of homogenization and pasteurization on viscosity has been carried on buffalo milk.

*Raw material:* Bulk buffalo milk from the experimental dairy of National Dairy Research Institute, Karnal was used in the experiments.

**Standardization:** Raw buffalo milk samples were standardized to 6 per cent fat with buffalo skim milk.

**Holder pasteurization:** Standardized milk was held in water-bath at 80°C so as to raise the temperature of milk to 63°C in about 5 min. Later, the milk was held in another waterbath maintained at 63°C for a period of 30 min.

**High heating temperature:** Standardized milk was held in a waterbath maintained at 80°C such that milk was allowed to come to 73°C within about 15 min and maintained at this temperature for 15 sec.

**Homogenization:** The sequence of homogenization was as follows: (i) Homogenization after pasteurization (HAP); (ii) Homogenization prior to pasteurization (HPP).

Milk was homogenized with Rannie piston type homogenizer, the pressures for the first and second stage being 180 kg/cm<sup>2</sup> and 40 kg/cm<sup>2</sup> respectively.

Standardized raw milk samples were preheated to 60-63°C before homogenization and then pasteurized (high heating temperature or holder). The second lot of milk samples were pasteurized (high heating temperature or holder) and later homogenized at 60-63°C. All samples were cooled and maintained at 4-6°C for a minimum period of 3 hr prior to determination of relative viscosity.

**Method of determining the relative viscosity:** Milk samples and water were tempered to 30°C for 10 min. Viscosity was measured with Ostwald viscometer for milk and water and ratio of viscosity of milk sample to viscosity of water was recorded as relative viscosity.

Thirteen replicates of each of the above treatments were statistically analysed by analysis of variance method.

It is observed (Table 1) that pasteurization, homogenization and the sequence of homogenization have significant effect in increasing the relative viscosity of milk. Puri and Gupta<sup>3</sup> recorded 3-4 per cent increase in viscosity of milk heated to 80°C for 5 min whereas in the present study the increase in relative viscosity was observed to be 3.5 per cent and 1.6 per cent for holder and high temperature pasteurization respectively. The greater increase in relative viscosity of holder pasteurized milk as compared to high temperature pasteurization can be attributed to higher drastic cumulative heat treatment in the former than the latter method which affects the hydration capacity of milk proteins.

The increase in relative viscosity on homogenization is attributed to the amount of fat surface, the amount of protein bound by the fat particles and the degree of clustering and clumping of fat.<sup>7</sup> Doan<sup>4</sup> and numerous workers have reported increase in viscosity of milk on homogenization. Single stage homogenization was more effective than double stage homogenization in increasing the relative viscosity of milk. It is due to the fact that the fat cluster formation is lower with double stage than with single stage homogenization. The relative viscosity of milk increased to a greater extent with HAP than with HPP treatment. This may be attributed to probable differences in homogenizing efficiency at the temperatures used in these treatments for homogenization.

TABLE 1. THE AVERAGE RELATIVE VISCOSITY OF BUFFALO MILK UNDER DIFFERENT METHODS OF PASTEURIZATION, STAGES AND SEQUENCE OF HOMOGENIZATION

Sequence of homogenization	Raw milk	Unhomogenized		Homogenized			
		Holder past.	High temp. heating past.	Holder pasteurization		High temp. heating	
				Single stage	Double stage	Single stage	Double stage
HAP	1.9425	2.0073	1.9765	3.6180	3.0510	3.5150	3.0268
HPP	1.7964	1.8662	1.8216	2.9359	2.6293	2.9087	2.6028
Average	1.8695	1.9368	1.8991	3.2720	2.8402	3.2118	2.8148
C.D.	...	...	0.2411	...	...	...	...
Per cent increase							
Overall	...	3.5	1.6	75.0	51.9	71.8	50.3
HAP	...	2.8	1.8	86.2	57.1	81.0	55.8
HPP	...	4.1	1.4	63.4	46.4	61.9	44.9

*F* test

Between treatments (6, 168)

Between sequence of homogenization (1,168)

C. D.: Critical difference; \* Significant at 1% level.

'F'

51.91\*

30.93\*



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## THE EFFECT OF FEEDING UNBAKED RAW MIXES AND HIGH-PROTEIN BISCUITS ON BLOOD PLASMA AMINO ACID LEVELS IN RATS

The effect of baking on the quality of high-protein biscuits was studied the blood plasma amino acid technique using weanling male albino rats fed diets at 10 per cent level of protein for a period of one week. Free plasma lysine levels were significantly reduced in rats fed test diets of biscuits compared to the values for unbaked blends. There was a marked elevation in the threonine, tryptophan and methionine levels in the plasma of rats fed biscuits and the scores indicated lysine to be the most limiting amino acid in the biscuits.

The blood plasma amino acid concentrations have been reported to be roughly proportional to the amino acid contents in the diet.<sup>1</sup> Consumption of a protein, deficient in a particular amino acid results in a reduced plasma level of the amino acid. This finding has

been applied for predicting the limiting amino acid in diets<sup>2</sup> and the technique provides a very useful index of the effect of processing on the *in vivo* availability of the amino acid. McLaughlan<sup>3</sup> observed that peak plasma lysine and methionine levels were much higher after a meal of alcohol-extracted fish solids compared to fish extracted with 1, 2-dichloroethane. Chicks fed overheated fish meal had lower concentrations of lysine and threonine than those consuming unheated fish meal<sup>4</sup>. In the previous paper<sup>5</sup>, the results of the effect of baking on the quality of protein in high-protein biscuits determined by the protein efficiency ratio (PER) method, were presented. Since the PER of biscuits was significantly lower than that of the unbaked ingredients, it became necessary to locate the cause of this reduction. In the present communication, the results of blood plasma levels of free lysine, methionine, threonine and tryptophan in rats as influenced by feeding high-protein biscuit diets are presented.

The two high-protein biscuit formulations designated A and B and the corresponding unbaked ingredients used in the present study were the same as those described in the previous paper<sup>5</sup>. The formulations of high-protein biscuits consisted of varying proportions of the supplementing protein-rich flours such as groundnut, soyabean, wheat germ and peas which per 100 parts of straight-grade wheat flour were: biscuit A, 20:5:10:10 and B, 20:25:0:0, respectively.

Weanling male albino rats were grouped into eight each, which were housed individually in cages with screen-bottoms. Test diets containing about 10 per cent protein ( $N \times 6.25$ ) and adequate in respect of vitamins<sup>6</sup> and mineral<sup>7</sup> requirements of the animals were prepared from unbaked ingredients, biscuits and skim milk solids, respectively. The level of fat in various diets was adjusted to 10 per cent with groundnut oil by allowing for the amounts already contained in the biscuits and the blends. The diets containing unbaked ingredients provided appropriate controls for studying the effect of baking on the plasma levels of the amino acids.

The animals were fed *ad libitum* for a week. Thick slurries of the test diets made with hot water were dispensed in individual porcelain cups. The food intake was restricted by removing the cups from the cages for six hours daily. This feeding regimen was adopted to induce the rats to start eating as soon as the diets were served after a short period of fasting. The rats were killed by decapitation after feeding

for six hours with the fresh diets, as in this period, the limiting amino acid decreases to a low level in the plasma.

To determine the fasting plasma amino acid levels, a group of 8 rats fed on the skim milk powder diet was fasted for 16 hr before being killed for taking blood for analysis. The concentrations of free lysine, methionine, threonine and tryptophan in the plasma of each animal were determined according to the microbiological assay procedure developed by McLaughlan *et al.*<sup>8</sup> The plasma amino acid scores were calculated according to the procedure described by Venkat Rao *et al.*<sup>9</sup> The results have been examined statistically by the Duncan's multiple range test<sup>10</sup> adopting square root transformation since the mean values were found to be proportional to the variance.

The effect of baking high-protein biscuits on the plasma levels of lysine, methionine, threonine and tryptophan and respective scores are presented in Tables 1 and 2 respectively. Data reported earlier<sup>5</sup> on the PER of unbaked ingredients and biscuits are also given for comparison.

TABLE 1. EFFECT OF FEEDING DIETS PREPARED FROM UNBAKED INGREDIENTS, BISCUITS AND SKIM MILK SOLIDS ON THE PLASMA FREE AMINO ACID LEVELS IN THE RAT

Gr. No.	Diet	Mean concentration, $\mu\text{g/ml}$ plasma $\pm$ S.E.*			
		Lysine	Methionine	Threonine	Tryptophan
I.	Unbaked ingredients (A)	26.8 $\pm$ 4.8	2.0 $\pm$ 0.2	12.3 $\pm$ 0.1	12.0 $\pm$ 1.3
II.	Biscuits (A)	8.3 $\pm$ 0.7	3.4 $\pm$ 0.2	48.3 $\pm$ 3.3	15.0 $\pm$ 1.1
III.	Unbaked ingredients (B)	28.5 $\pm$ 4.2	2.4 $\pm$ 0.2	9.7 $\pm$ 0.6	12.0 $\pm$ 1.4
IV.	Biscuits (B)	9.4 $\pm$ 0.8	3.5 $\pm$ 0.2	51.2 $\pm$ 3.0	20.0 $\pm$ 1.3
V.	Skim milk powder	85.3 $\pm$ 4.4	8.0 $\pm$ 0.5	75.7 $\pm$ 6.4	20.2 $\pm$ 1.3
VI.	None (fasted)	88.0 $\pm$ 6.1	6.2 $\pm$ 0.5	79.8 $\pm$ 5.1	14.7 $\pm$ 1.2

Groups: I III    II IV    V VI                      II IV    I III    VI V

Threonine                                      Tryptophan  
 Groups: IV II    I III    V VI                      IV II    VI I    III V

Note:—Means not underscored by the same line are significantly different.

Means underscored by the same line are not significantly different.

\* Standard error

TABLE 2. PLASMA AMINO ACID SCORES AND PROTEIN EFFICIENCY RATIO OF UNBAKED INGREDIENTS AND BISCUITS

Gr. No.	Protein source in diet	Plasma amino acid scores				PER	S.D.* ( $\pm$ )
		Lysine	Methionine	Threonine	Tryptophan		
I.	Unbaked ingr. (A)	30	32	15	82	2.18	0.10
II.	Biscuit (A)	9	55	61	102	1.41	0.13
III.	Unbaked ingr. (B)	32	59	12	82	1.99	0.15
IV.	Biscuit (B)	11	56	64	136	1.55	0.14

\* Standard deviation; Data for PER from Ref. 5

The plasma lysine values were significantly lower in rats fed test diets prepared from biscuits A and B compared to the diets of unbaked ingredients. The results clearly establish the poor availability of lysine in the biscuits as a result of the formula which included malt and corn syrups and baking conditions. The threonine levels in the plasma were much higher while methionine and tryptophan contents were slightly higher in the animals consuming biscuit diets than the controls. There appears to be an accumulation of methionine, threonine and tryptophan in the blood of rats as a consequence of reduced availability of lysine. Marked elevation in the threonine level in the blood of rats and chicks<sup>11,12</sup> when lysine was limiting has been reported. Threonine may be deaminated relatively slowly as compared with the other amino acids and hence may accumulate in the blood as a consequence of reduced availability of lysine. The plasma amino acid scores (Table 2) emphasize lysine to be most limiting in both the biscuits while lysine, threonine and methionine appear to be co-limiting in the unbaked ingredients as well.

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THE NATURE OF MAJOR PROTEIN COMPONENTS OF THE NEWER VARIETIES OF *CICER ARIETINUM*

Six varieties of *Cicer arietinum* (Bengal gram) were analysed for their total protein (24-28 per cent) and their non protein component (12-15 per cent). 0.4 M sodium chloride soluble protein of these varieties were dialysed to separate the globulin (60-80 per cent) and water soluble fraction (3-4 per cent). The globulin fractions were analysed by Sephadex (G-150) gel chromatography (two components 80 and 20 per cent), sedimentation analysis (S<sub>20</sub> w 8 and 13) and electrophoresis on polyacrylamide gels in presence of 8M urea. Although the globulin fractions of the different varieties behave similarly on gel chromatography and sedimentation analysis, there were distinct differences as revealed by acrylamide gel electrophoresis.

*Cicer arietinum* (Bengal gram) is the major legume of India and is extensively used in a wide variety of food formulations. Preliminary work on the proteins of the Indian varieties of Bengal gram was reported several years ago<sup>1,2</sup>. Newer high yielding varieties of the legume are being developed and cultivated in India<sup>3</sup>. It was felt that a comparative study of the nature of major proteins from the newer varieties with modern separation methods was necessary.

Bengal gram flour (80 mesh) was defatted in a Soxhlet apparatus with petroleum ether (40-60°C range) till the extract was colourless (6-7 hr). It was later extracted with 0.4 M sodium chloride (10 per cent W/V) at room temperature for 30 min and the suspension centrifuged at 5600 g for 30 min at 4°C. The extraction was repeated twice and the combined supernatants dialysed against 70 volumes of distilled water at 4°C until chloride free (about 72 hr). The precipitated protein was collected by centrifugation and freeze dried. This constituted the globulin fraction and was used for all subsequent studies. Non protein nitrogen was determined in the supernatants of 0.4 M sodium chloride extracts adjusted to 5 per cent (W/V) trichloroacetic acid concentration and keeping for 16-18 hr at 4°C. Nitrogen was determined by the micro-Kjeldahl method and a factor of 6.25 was used to convert the nitrogen into protein.

The results obtained with different varieties are given in Table 1. The total protein content showed little variation (24-28 per cent) among the varieties. Nearly 85-90 per cent of the nitrogen was extractable with 0.4 M sodium chloride in most of the varieties except variety 'BG-482' where only 73 per cent was recoverable. Gel filtration on Sephadex G-150 (2.7 × 57 cm column loaded with 100-150 mg protein) using 0.005 M sodium borate buffer, pH 8.6 as eluant indicated the presence of two main components eluting at *V<sub>e</sub>/V<sub>o</sub>* of 1.295 and 4.00. The first component represented nearly 80 per cent of the protein applied and the second component about 20 per cent. The chromatographic behaviour of all the varieties was very similar. Chromatography of the globulins on DEAE-Sephadex-A-50 (1.3 × 10.5 cm column loaded with 35-40 mg protein) using 0.005 M sodium borate buffer, pH 8.6 with stepwise salt gradient 0-1 M sodium chloride resolved them into four fractions which were

TABLE 1. EXTRACTION OF PROTEIN FROM DEFATTED BENGAL GRAM FLOUR

Variety	Total N g/100g flour	Soluble N as % of total N			Total N extrac- ted %	Non- protein N %
		1st extr.	2nd extr.	3rd extr.		
T-3	3.97	71.6	13.8	4.2	90	11
MP-58	4.13	70.6	11.5	3.9	86	14
BR-77	3.80	74.3	12.3	4.4	90	15
BG-482	4.44	52.0	14.5	5.9	73	15
ST-4	4.46	65.6	12.9	5.2	85	14
G-24	3.97	68.9	14.6	6.4	90	12

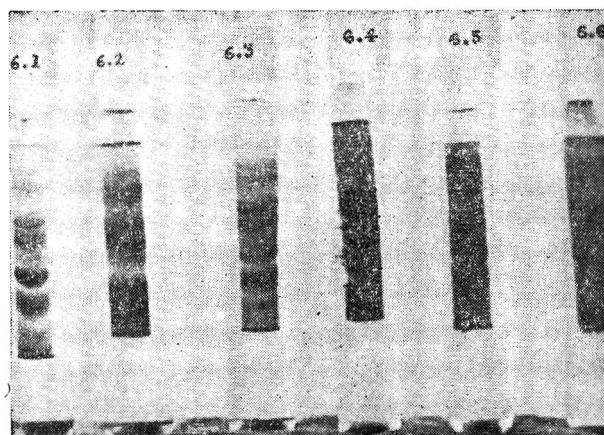
still heterogenous by acrylamide gel electrophoresis. In case of variety 'G-24,' acrylamide gel electrophoresis of the total globulins at pH 8.3 in the presence of 8 M urea revealed eight bands. The first DEAE-Sephadex fraction (borate buffer 0.005 M, pH 8.6, only 60 ml eluant) contained 45 per cent of the total protein (4 bands), whereas the subsequent ones, buffer with 0.2 M NaCl, 50 ml eluant had 14 per cent protein (5 bands), with 0.3 M NaCl (50 ml eluant) had 13 per cent protein (6 bands) and with 0.5 M NaCl (50 ml eluant) had 6 per cent protein (number of bands uncertain). Sedimentation analysis<sup>4</sup> (1 per cent solution of globulin in 0.1M sodium borate buffer, pH 8.6) again revealed two main components with  $S_{20,w}$  value of nearly 8 and 13 in nearly all varieties except T-3 (Table 2). In the presence of 8 M urea the globulins (variety 'MP-58') dissociate as revealed by sedimentation ( $S_{20,w}=2$ ). Disc electrophoresis of the total globulins at pH 8.3 (tris-glycine buffer) show some differences but the bands are diffuse. However in 8 M urea at different pH values<sup>5,6</sup> the globulins resolve well on gel electrophoresis and as many as 17-22 bands (pH 3.8 in presence of 8 M urea) were visible after staining (Fig. 1).

The two major components isolated by Sephadex gel chromatography and also revealed by sedimentation analysis are most probably similar to vicillin and legumin in agreement with Danielsson<sup>7</sup> who examined many legumes. The amino acid composition of vicillin and legumin derived from *Cicer arietinum* have been reported earlier<sup>8</sup> and are comparable in composition to similar fractions derived from other legumes<sup>9</sup>. Evidence that legumin and vicillin of *Cicer arietinum* consists of several subunits with different N-terminal groups has been shown but still there is a considerable amount of similarity in legumin and vicillin as revealed by peptide maps<sup>10</sup>. It is proposed to determine at a later date the amino acid composition of the two major components as well as compare the peptide maps of vicillin and legumin from the different varieties.

TABLE 2. SEDIMENTATION ANALYSIS OF TOTAL GLOBULIN OF DIFFERENT VARIETIES

Component	Sedimentation coefficient ( $S_{20,w}$ ) of different varieties					
	T-3	MP-58	BR-77	BG-482	ST-4	G-24
A	9.5	13.0	13.2	13.1	12.7	12.8
B	8.2	7.8	8.3	8.4	7.4	7.4

Component 'A' is probably legumin and 'B' is vicillin. Beckman model E-analytical ultracentrifuge was operated at 59780 rpm.



(-)  
T-3 19 MP-58 19 BR-77 22 BG-482 19 ST-2 17 G-24 17

FIG. 1. Polyacrylamide gel (7.5%) electrophoresis of total globulins (each 200 $\mu$ g) of different varieties of Bengal gram at pH 3.8 (glycineacetic acid) in 8.0 M urea; stained later with amido-black, destained with 7% acetic acid and photographed. The photographs were scanned in a Chromoscan microdensitometer made by Joyce-Loebl Ltd., Gateshead, U.K. Filter No. 626 with reflection attachment was used. The number of distinct peaks recorded represent the number of bands.

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## BOOK REVIEWS

*Evaluation of Mercury, Lead, Cadmium and the Food Additives Amaranath, Diethylpyrocarbonate and Octyl Gallate.* W.H.O. Food Additive Series No. 4, World Health Organization, Geneva, p. 84.

This is a publication of the World Health Organisation, prepared by the joint FAO/WHO Expert Committee on Food Additives which met in Geneva on 4-12 April 1972. The book presents monographs on mercury, lead, cadmium, amaranath, diethyl pyrocarbonate and n-octyl gallate. These monographs cover methods of analysis, biological data, long-term and short-term toxicity studies, assessment of hazards to man and evaluations with specifications, written by experts in the field, the data included in the book provide most valuable guidelines to ensure the safety of foods. This is a very useful compilation not only to a laboratory worker on toxicology but also to those who are the custodians of public health. This book has the merit of serving as a day-to-day guide to food safety. Hence this will be a very valuable addition to the library.

V. SREENIVASA MURTHY

*Aspects of Meat Inspection:* by H. THORNTON, Bailliere Tindall, London, 1973, pp. 172. Price £ 3.00.

This book written by an experienced international authority is a welcome departure from the usual manner of presenting meat hygiene. Equal emphasis has been given to various handling factors not necessarily physiological as well as disease and veterinary factors that affect meat quality. The presentation is divided into 10 chapters.

The administration of tranquillizers to animals before transport from the farm to the abattoir has to be viewed with caution because of the possibility of undesirable tissue reactions and the possibility of residues. The meat from a tranquillized animal has a lower ultimate pH which is definitely desirable from the point of preservation. Discussing the effect of fatigue and starvation, it is opined that the bone taint is due to bacteria from the gut. Taint of muscle is definitely bacterial and the pH of meat has a large bearing on susceptibility to bone taint. But the loci of bacteria responsible for bone taint are lymph

glands. The industry involved in conversion of muscle to meat is faced with some conflicting facts: (a) fatigue can be offset by resting, (b) during this resting the animals are exposed to cross-contamination of potentially dangerous infection, particularly, salmonellae and the duration is not enough to produce symptoms or lesions detectable at anti- and post-mortem examination, and (c) feeding prior to slaughter, particularly in pigs, has favourable points from bacon quality, but may increase the number of bacteria infiltrating into blood circulation from the guts. Feeding of readily absorbable and assimilable sugars to pigs 3 to 4 hours before slaughter can be used to shorten the detention period in liarages.

The primary recommendations for slaughter are (i) adequate rest, (ii) stunning to make it unconscious, (iii) complete bleeding in a vertical position, (iv) prompt and careful evisceration, and (v) prompt chilling. A worthwhile suggestion is that the state of *rigor mortis* of heart observed during post-mortem examination may be an index of the rest before slaughter. Bleeding in a horizontal position has advantages of preventing internal bruising of the ham.

In the chapter on post-mortem examination, the various conditions of liver abscesses, colour and enlargement that are encountered are dealt with rather than the routine examination which is well described in all text books. Similarly in describing meat quality tenderisation by injection of papain solution is dealt with. Intravenous injection of purified papain solution 5 to 30 minutes before slaughter in amounts depending on weight of the animal which is restrained in a special 'crush' gives optimum tenderisation. In a normal carcass about 35 per cent of the cuts are tender enough for roasting. But in a carcass from preslaughter tenderised animal, 75 per cent of the cuts are suitable for roasting. This type of preslaughter tenderising is particularly effective in animals which are beyond the age to yield physiologically tender meat. The interpretation given to the work of Meat Research Industry Institute in New Zealand on cold shortening is that it is preferable to chill the carcass after dissipating body heat.

Discussing dark-cutting beef it is said that the mechanism of production of this phenomenon is incomplete oxygenation of myoglobin. While this

is one of the reasons, the influence of water held free or bound and the affect of this on structure and reflection of light is far more important. PSE or watery pork is due to a rapid post-mortem fall of pH of the muscle, but the explanation given that it is due to accumulation of lactic acid due to conditions before slaughter is not correct.

Four chapters are devoted to discussion of pathological lesions observed during the author's experience and cover liver cirrhosis, telangiectasis, foreign bodies in the bile duct, torsion of the spleen, a slaughter testicle in the crypto-orchid pig, salmonellosis and parasitic diseases.

The book is a worthwhile addition to libraries catering to post-graduate and scientific workers interested in meat quality.

B. R. BALIGA

*Edible Coatings and Soluble Packaging:* by ROGER DANIELS, Noyes Data Corporation, Park Ridge, New Jersey, U.S.A. 1973, pp. 360. Price £36.

The desire for convenience in the preparation of foods and the dominance of consumerism in marketing newer foods lead to the development of ready to cook and ready-to-serve foods. In consonance (concomitant) with this is the development of convenience packages which apart from providing protection allow cooking and/or serving the food in the package itself and discarded thereafter. Further advance in this direction is the development of soluble and edible package which can be cooked and eaten as integral part of the food. For many of the drugs it is essential to encapsulate them in edible films or coatings for dispensing in the required dosages. Ready-to-cook or -serve foods are generally mixtures of various food items differing in moisture contents, texture, flavour, etc. There can be deleterious interaction between some of them due to transfer of moisture and colouring matter and diffusion of flavours etc., and they need to be protected from one another within the mixture. This necessitated the development of edible coatings to isolate the desired constituents from others to protect them from moisture pick up, oxidative rancidity, disintegration, etc. Some of the coatings which form strong films are very efficient where the film has to form a skin tight package as in the case of meat chunks for frozen storage. Without these coatings packaging of such foods might be impossible or expensive. The book 'Edible

Coatings and Soluble Packaging' under review is a good source book for those developing such foods and looking for edible coatings and packages.

This book is a review mainly of U.S. patent literature since 1960 as claimed by the author. As the development of such foods in their divergence has taken place mainly in the U.S. the patent literature on the subject in U.S. is the largest and comprehensive and this book reviewing these patent literature can also be expected to be comprehensive. Drawn from the patent literature the information contained can be taken to be somewhat reliable. Though the information is drawn from patent literature it is free from the legal jargon but without leaving significant information. Because the patent information has been presented in a condensed form sometimes the text tends to be vague even though such essentialities like critical values of concentration, pH, temperature are mentioned, but the examples that follow, clarify the processes described. Wherever a patent apparatus is described, line diagram or sketches are given. The discussion on almost all the processes contain a brief information on the disadvantages associated with the earlier techniques and how the patented process described overcomes the problems.

The information is divided into ten chapters of which the last nine deal with the application to different category of foods.

The first chapter deals with general descriptions of preparation of the main types of edible film and coat. Under each type of coatings different methods of forming them are reviewed. Under amylose films and coatings the methods and applications given are: coating films by coagulation from caustic solution and also from solution using salt and acid; chemical treatment and autoclaving methods for preparing dispersible amylose to be used for casting films; surface treatment of amylose films to improve tensile strength, flexibility and water insensitivity; its application as surface coating to potato products; preparation of starch slurries suitable for water soluble coating and a method to coat the starch film on cereals, fruits, cheese, sandwiches, eggs, etc., with an illustration and description of the apparatus for the purpose. Under the collagen films, preparation of enzyme modified collagen dispersion from which collagen films can be cast, and their application to meat snacks by suitable variations in the process are described. Under cellulose coatings, ethyl cellulose compositions with mineral oil and with plasticizers and cellulose ether compositions with film suitable for hot coating on foods are given.

The section on protein coating covers: a method of extraction and isolation of keratin from natural sources; two processes for the preparation of films and coatings from keratin and wheat gluten; modification to the processes by addition of particulate silicates to improve deficiencies in the processes and a method for forming heat sealable and soluble protein films which can be made into pouch form suitable for holding about an ounce of dry foods.

The second chapter is on fruit and vegetable coatings. Coatings for fruits and vegetables are mainly to minimise desiccation and deterioration due to bacteria and fungi. For this purpose lecithin emulsion coating containing antibacterial and fungal agent for reducing decay due to microorganisms; synthetic plastic coatings and dry coatings using carboxymethyl cellulose derivatives to reduce rate of respiration and desiccation; and wax resin or oil coating for reducing rate of respiration and to obtain high gloss on the fruit surface are described. Apparatus for spray coating and also for gloss coating with wax, etc., are illustrated. A method for coating sauce or juice on quick frozen discrete food pieces is described with the aid of a flowsheet.

In the next chapter coatings for raisins, dates, and other sticky foods like confectionaries are described. The main function of the coatings for these products is to protect them from gaining or losing moisture and sticking to each other and forming a clump. For wax coating of raisins two USDA processes one for controlling the thickness of the coating and another for cooling after coating are well illustrated. Vinyl polymer coating and polysaccharide coating with an over coat of wax are described for raisins. Amylose with plasticizer as coating for dates, figs, dried fruit and candied fruits, and a sterilization and coating method for long term storage of dates are described. The processes of precoating corn syrup on fruit segments before drying or after freeze drying and the amylose fatty ester/protein laminate coating on food products for protection from moisture and oxygen would go well under fruits and vegetables coatings than under the coatings for sticky products. Similarly the section on solid fat coating on dried fruits for cake mixes and alginate or carrageenane coatings on candied fruits can be better included in the chapter on nut coatings. For confectionery a coating of water-in-oil emulsion as a substitute to regular fat coating is discussed in good detail. A process though not of coating, for reducing stickiness of candy by adding a nonhygroscopic polyethylene

glycol to the hot candy mass is also touched upon. At the end a method to form a protective chocolate coat on dehydrated gels is also included.

In the fourth chapter coating for nut products are reviewed. Edible coatings for cooked or roasted nuts are useful not only for protection against oxidation and moisture absorption but also for binding salts, flavours, colours and preservatives. They are particularly useful for nuts added to wet foods like ice cream in retarding moisture uptake. The processes for protection described include: roasting and gum-arabic coating of nut meats; sorbitol-mannitol mixtures and gluten-dextrine coatings for roasted peanuts; simultaneous roasting and hexitol coating of nuts; plastic edible shortening coating of nut meats with simultaneous sterilisation; and coating with pre-gelatinised dry starch and fusing it into a film on the nut meat. Two methods for colour coating of nut shells one of which describes the sealing of cracks in walnuts and lists of F.D.A. approved walnut colouring formulae are also included.

The next chapter on Meat and Fish Coatings reviews many edible coatings for fresh and processed meat and for fish. The author says that the literature on artificial casings is extensive and will be dealt in a separate volume. In this chapter only approaches for their preparation and use are covered. The edible coatings for meat and meat products are to protect them from colour loss, freezer burn, fat rancidity, and mold or bacterial growth. For fresh meat the coatings discussed are: gelatin—propylene glycol for protecting primal cut or whole carcasses at freezer temperatures; alginate protective coating including methods to minimise browning during cooking and a method to remove coating for inspection; coating of fatty film by simple application and solidification of water-in-oil emulsions on meat to be stored at low temperatures; coating of mono- and triglycerides on meat to prevent dehydration and the outflow of meat juice after slaughtering and cutting; and coating of ethyl cellulose plasticized with vegetable oil for frozen meat to prevent moisture loss and odour transfer. The necessity of surface oxidation of meat prior to coating with ethyl cellulose is not justified and instead oxygenation might be more appropriate. For meat products like sausages the coatings included are: gelatin-metaphosphate for dry sausages; gelatin of metal for salami sausages; alginate film coating on extruded sausages; permeable film coatings to sausages for allowing proper evaporation; and collagen coatings, made stable by the treatment of glyceraldehyde, for hamburgers. For fish and other sea foods the coatings include a coating of edible starch

water soluble algin mixture applied after inactivating enzymes and bacteria, and mixture of mono- di- and triglycerides as film coatings on fish fillets for preventing dehydration and deterioration.

In the sixth chapter eleven processes of coating cereal products such as potatoes and rice products are reviewed. These coatings are primarily for the protection of quality of the product, and when modified can also improve texture. Surface treatment of ready-to-eat breakfast cereal flakes with hydrophilic silica materials retard softening when milk is added to them and a coating of pregelatinized starch-fat on puffed cereal will give a taste, texture and appearance as a nut. Candy coating on cereal nut mixtures, after precoating them with a binder gum, eliminates the separation of cereal from the nut when added to milk. Coating of edible calcium salts instead of traditional talc powder on milled rice imparts necessary glaze. Coating of sugar and starch to instant rice pudding reduces their separation from the product. Flavour can be solubilized in dry coating on rice grains and leaching of amino acids from fortified artificial rice can be prevented by gelatin/starch coatings.

Coatings for bakery products are discussed in the next chapter. The principles and methods are essentially the same as for coatings on cereals. Coatings for bakery products reviewed are: film forming dispersions, coatings for food bars, glazes for doughnuts, dusting powders, and fat coating for ice cream cones and baked goods of low moisture content to preserve crispness. Processes other than coatings for finished products included are: preparation of free flowing baking mixes by spraying shortenings; a method for producing a non sticky layer of bonded icing dry mix to facilitate easy pouring of and removal of cake mixes from polyethylene bag. A method for forming tubular sponge cake within a cardboard sleeve and making of edible double wall container from materials like pastry for hot foods are described.

Encapsulation process is the subject of the eighth chapter. Encapsulation of a single dose of a product is an important form of edible packaging particularly for drugs but in food only those ingredients which are unstable or volatile are encapsulated. A basic process for encapsulating microscopic oil droplets consists of steps of emulsifying the oil with a hydrophilic colloid solution and addition of aqueous solution of another colloid with opposite charge and cause deposition of the complex colloid around individual oil droplets by causing coacervation by dilution or pH adjustment. An extension of the process for multiple wall (film) encapsulating is also given.

A different method for directly encapsulating a filler material is described with schematic diagram of the equipment. Protein or non-protein based materials capable of forming aqueous hydrophilic colloids can be used for encapsulating oils by emulsifying them together and gelling the colloid either by change in temperature or concentration. Similar processes using gluten and dextrin derived from oxidised starches instead of protein are outlined. Fat coating of flavouring nucleoside-5'-phosphates in granular form, for protection from the enzymic action of phosphatase present in the main food, is described.

A simple method of fixing flavouring materials into the matrix of flavour powder particles and another for fixation of acetals by forming complexes with dextrin and acetaldehydes in a matrix of manitol solids are described. A process for packing instant soluble coffee in gelatin capsules and the advantages of perforating the capsule with a hot needle is illustrated with diagrams.

The ninth chapter is on Coatings for salt-type ingredients and vitamins. Common salt acts as prooxidant when present in a crystalline form in wet foods. Other food ingredients like spices etc. deteriorate by themselves or due to the moisture uptake from the main food constituents. Edible coatings to prevent these effects discussed are hydrogenated fat or molten curing salt coatings for salt and ethyl-cellulose coating for salt, pepper and other condiment; and prolamin film coating for meat curing agents; and fat emulsion coating for salt and sugar. The coatings for vitamins described are gelatin emulsion, saturated fatty acids and amylose emulsions.

The last chapter deals with Miscellaneous coating preparations and applications. Calcium sorbate coating on the surface of hard cheese or hard sausage prevents the formation of mould fungi but without preventing the permeation of air. The process given under coating fibrous protein is nothing but an impregnation vegetable oil in toasted textured protein. To eliminate caking of soyabean meal during storage, coating of all the particles with a dusting powder like kaolin containing small amount of fat, after toasting the product and grinding the oversize particles to a desired size, is said to be expensive than other methods of simple dusting. Fat-starch (dextrinised) coating for animal foods for retarding fat oxidation and protection from crumbling is indicated. Alginate gel for better coating adhesion for frozen cooked foods and the formation of a gel like skin on extended form of foods are included. A process for removal of algin coating on foods using calcium sequestering agent like hydroxy acids is



intended mainly for inspection purpose of the food coated. A gelatin membrane is suggested as a partition between acid fruits and sour cream, etc. in a single container to avoid deterioration of the cream.

The book also contains a content-cum-subject index in the beginning but without numbering the chapters and at the end it contains three indexes: company index, inventor index and patent number index, and finally a notice cautioning the reader on the use of information contained in the book.

As claimed by the author, the book, by presenting an advanced and commercially oriented review of edible coatings and soluble packagings, serves as a source of technical information and also as a guide to the U.S. Patent literature on the subject. An indication of the probable deficiencies and economics of the processes discussed could have added further to the value of this review to serve as a guide.

P. VEERRAJU

*Low Temperature Preservation of Foods and Living Matter:* by OWEN R. FENNEMA, WILLIAM D. POWRIE AND ELMER H. MARTH, Marcel Dekker Inc., New York, 1973, pp. 598.

The book is written by three well known scientists with considerable knowledge and experience in the subject matter. There are eleven chapters dealing with various aspects of low temperature preservation of foods and living matter.

The first chapter opens with the remark that the only substance on this planet that occurs in all three physical states is water and water content in various foods and biological materials is one of the most important factors to be considered while freezing the product. Freezing, in its simple form, involves a temperature reduction coupled with removal of water from solution in the form of pure crystal. The author while describing the physical properties of water and ice adds that water solute interaction in biological matter can influence a variety of essential functions, including the mobility of protons and ions, the reactivity of proteins and the structure of biological membranes, proteins and other biopolymers.

The second chapter includes topics on problems of freezing preservation and discusses the nature of food systems and their relation to living matter; comparative microstructure and metabolic feature of several cell types commonly preserved by freezing and thermal properties of foods and living matter. The author has classified foods into four broad categories namely

(i) essential intact cellular systems, e.g. whole fruits, vegetables, eggs, meat; (ii) disrupted complete cellular systems, e.g. ground meat, purees, flour, soup; (iii) non cellular or disrupted incomplete cellular systems e.g. milk, fruit juices, oils, corn syrup, sugar, honey; or (iv) combinations thereof, and further places emphasis on the need for thorough acquaintance with colloidal systems, cells, tissues, and the physical and chemical changes they undergo during freezing. In addition to above, thermal properties of foods and living matter have been dealt in detail to enable calculation on rates of and energy requirements for freezing and thawing. All these studies become necessary to understand the mechanism of damage in different food systems and animal matter during freezing and thawing.

The third chapter on solid liquid equilibria presents phase equilibria and freezing curves for pure water, binary aqueous systems and for foods and living matter. This chapter also deals with freezing point depression and determines the freezing points from time temperature plots. Instruments for determination of freezing point is also described.

Chapter four deals with freezing, frozen storage and thawing of foods and living matter. In the first part the author examines the physical and chemical consequences of freezing. In the second part, the author records the physical and chemical changes associated with the products during frozen storage and in the third part, describes the nature and rate of thawing as compared with freezing. Thawing procedures deserve considerable attention as it is a well known fact that the rapid thawing is essential for retaining viability of many kinds of biological specimens and also for retaining the quality of some foods.

The next two chapters, five and six deal with cryopreservation of milk and milk products; egg yolk, and food myosystems. Interesting information on the changes in the physical and chemical properties of lipids in frozen milk and milk products; in rheological properties of yolk during freezing and thawing and chemical changes in muscle during frozen storage have been presented.

The seventh chapter presents classification and structures of edible plant tissues and describes changes in structure, texture and chemical composition of plant tissues during freezing and frozen storage. Some of the causes for off flavours, off odours, chlorophyll deterioration, ascorbic acid oxidation and changes in pH in plant tissues are stated.

Chapter eight covers the portion on the behaviour of food microorganisms during freeze preservation. The author lists the causes and kinds of cryo-injury to microbial cells and suggests some remedial measures to minimize injury. Microbiology of frozen foods such as ice, milk, milk products, fruits, fruit juices, vegetables, eggs, poultry, meat, seafoods and pre-cooked frozen foods is also dealt here in great detail. The author points out that the data related to manufacturing practices in the plant in order to determine what can be achieved when the food is processed under optimal conditions, if made available, can immensely benefit those who are engaged on developing and perfecting the microbiological standards for different foods.

Chapter nine discusses the method of freezing preservation of animal cell suspensions (human red blood cells, spermatozoa, bone marrow and cultured animal cells) and organized animal tissue (human skin, cornea, whole mammalian organs) and recommend the uses of cryoprotectants such as glycerol and penetrating chemicals prior to freezing. However, storage at  $-80^{\circ}\text{C}$  or at lower temperature is necessary and after removal from storage, rapid to very rapid thawing is imperative. The tenth chapter advocates the application of cryoprotectants to living specimens prior to freezing and describes the properties of various cryoprotectants available. The author discusses the mechanism of freezing damage to the cells and has put forward some of the hypothesis which need further investigation. The last chapter

presents the technological aspects on freezing preservation of foods, however, the subject matter dealing with freeze drying has not been dealt with. The methods and equipment for freezing foods by air freezing (conventional and fluidized bed), plate freezing, cryogenic freezing are described. The cost of freezing by various methods has been given and is shown to depend upto the large, uniform rate of production. The total cost of freezing is determined by not only fixed and operating costs but also by the amount of water lost from the product during freezing. The air blast and fluidized bed freezing are the least expensive. This chapter also includes information on the prefreezing treatments of vegetables, fruits, fish, seafoods, poultry, meat, eggs, milk, precooked foods, etc. The storage of frozen foods and their loss in quality are also presented. Suitable methods of thawing are discussed.

The Book contains most valuable information on low temperature preservation of foods and living matter. The book will serve the interest of those who are engaged on research in food technology, microbiology, medicine and commercial freezing operations. It deals at great length on some of the practical applications of freezing preservation techniques to foods and biological materials and suggests some practical methods of thawing the frozen materials without loss of quality. Students of food science will find this book very useful and easy to grasp.

V. B. DALAL

### **Abstract Bibliography on Dioscorea**

The Ford Foundation, New Delhi-3, Indian Institute of Horticultural Research, Bangalore, and IARI, New Delhi have jointly brought out the above publication. There are 279 references included and covers the period from 1931 to 1972. The references were collected from Biological Abstracts, Horticultural Abstracts, Chemical Abstracts and other unpublished sources. Although this is a food crop its pharmacological value is well known and it will prove a valuable source material for those engaged in research and also in reviewing the past work and knowing the existing gaps in our knowledge about this valuable plant material. The publication will be available on request free of cost with Indian Institute of Horticultural Research, 255, Upper Palace Orchards, Bangalore-6.

### **Xth International Congress of Nutrition**

The Xth International Congress of Nutrition, sponsored by the International Union of Nutritional Sciences (IUNS), will be held in Kyoto, from August 3rd to 9th, 1975 at Kyoto International Conference Hall.

The Science Council of Japan, member of IUNS, is the host organization of the Kyoto Congress. About 2,500 professionals in the field of nutritional sciences from all over the world are expected to take part in the Kyoto Congress.

The scientific programme committee of the Xth Congress has been planned to allot about a half of the time available to Free Communication and the remaining to Congress Symposia.

Volunteer papers for oral presentation will be selected by the Programme Committee on the basis of scientific merit, relevance to the programme and variety of subject matter. A list of topics for the papers of Free Communication will be released in the 2nd circular of the Congress.

Congress Symposia will be held simultaneously in the following 4 Sessions:

- A. Recent findings in Basic Problem of Nutrition
- B. Problems in Clinical Nutrition
- C. Foods for the Present and Future Demands
- D. Approaches to Dissolve the Present Problems

On submission of contributed papers authors will have to submit summarized text written in English, which will be used as a basis for the selection and classification of papers, and also as work documents at the Congress Sessions.

All correspondence should be addressed to: Xth International Congress of Nutrition, C/o. Kyoto International Conference Hall, (Cable, Inthall Kyoto: Telex. 5422353 Inthal J), Takara-ike, Sakyoku, Kyoto 606, *Japan*.

### **VIth International Congress of Essential Oils**

This will be held in September 8th-12th, 1974, at San Fransisco, California, U.S.A. The Congress Programme will be in four main sections: Agricultural/Botanical, Chemistry/Technology, Utilization of Essential Oils and General Business Sessions.

Congress co-chairman James Rogers, who is organizing the technical programme, indicates that a wide spectrum of papers from leading international figures in the essential oil industry will be presented at the Congress, covering technical and scientific achievements based on original research work in the field of essential oil technology. The Congress programme will include the most complete and up-to-the-minute reports on recent scientific developments in essential oils, essential oil chemistry, essential oil agronomy, and related scientific topics.

For further information write to: VIth International Congress of Essential Oils, 60 East 42nd St., New York, N.Y. U.S.A.

### **IVth International Congress of Food Science and Technology (Madrid, September 22-27, 1974)**

#### *A. Student Participation*

The Organization Committee of the IV Congress, following the precedent established by previous Congresses, has attempted to encourage the presence of students. For this reason, some special economic conditions have been established and a special programme organized, of particular interest to students.

The Symposium 'Students and Food Science and Technology' occupies a prominent position in the programme and which will be held in two Sessions. The first of these will be dedicated to the subject,

'The professional vocation for food science and technology', and the second to 'Professional activities in food science and technology'.

The students will be permitted to participate as they choose in all activities of the Congress, according to their interests; among these, one of special importance is the round-table Session directed by Dr Kefford concerning 'Curriculum and texts for the theoretical and practical teaching of food science and technology'.

The Organizing Committee of the Congress grants to students exemption from the payment of inscription and invites them to participate in the excursions planned for the free day of the congress, and in the closing dinner; *also, reduced prices for room and board are offered.*

The forms and conditions of inscription may be obtained from the Secretary of the Congress.

#### *B. Preliminary Programme and Round Table Meetings*

The Congress Preliminary Programme has been published, and can be requested from the Congress Secretariat. It gives, in detail, a description of the different aspects of the programme.

The Scientific Programme Committee has received more than 400 papers for the selection of those to be read during the Congress. At present, this committee, along with the Presidents of the sessions, is dedicated to their study and selection. The most popular subject (more than 30 papers received) have been the following: 1a, Chemical constituents of foods related to flavour odour, and texture; 5a, New methods for the conservation of foods; and 8a, New sources of foods rich in nutritive substances.

In the scientific aspect, four days are devoted to the working sessions, consisting of the presentation and discussion of the selected research papers, and the round-table meetings; attendants wishing will also be able to participate in several technical visits.

#### *C. Round-Table Meetings*

As it has already been announced, the scientific programme of the Congress will include two types of sessions: those devoted to the presentation of research papers, and seven round-table meetings. Titles and chairman of the latter have been definitively settled as follows:

1. Current experience of waste treatment in the food industry. Dr A. I. Morgan, Director, Western Utilization Laboratory, U. S. Department of Agriculture, U.S.A.

2. Techniques in the forecasting of food research needs. Prof. J. Hawthorn, Head, Department of Food Science and Nutrition, University of Strathclyde, U.K.
3. Recent achievements in the utilization of vegetable protein: (a) oilseed protein, (b) cereal protein, (c) leaf protein and protein from other sources. Prof. N. S. Scrimshaw, Head, Department of Nutrition and Food Science, Massachusetts Institute of Technology, U.S.A.
4. Education and training in Food Science and Technology: curricula and text books, Dr J. F. Kefford, Assistant Chief, C.S.I.R.O., Division of Food Research, Australia.
5. Criteria for the evaluation of the consumer's needs as a basis for the planning of research and development in Food Technology, Dr A. S. Clausi, Vice-President, Corporate Research Department, General Foods Corporation, U.S.A.
6. Documentation and Information. 6a. Current problems of the Food Science and Technology Journals. 6b. Documentation and information in food industry. Dr G. Dardenne, General Secretary, French Committee for Science, Technology and Economy of the Food Industries, France.
7. Cooperation between research centres and international organizations for transfer of technology to develop food industries. Dr H. A. B. Parpia, Senior Officer, Agricultural Services Division, F.A.O. Headquarters, Rome-Italy.

Round-table meeting will start by a board presentation of the topic by the Chairman, followed by the presentation of the specific aspects to be treated, each by the corresponding reporter appointed by the Chairman, if he thinks it necessary.

If interested in receiving further information please write to:

Secretaria del IV Congreso Internacional de, Cienciay Tecnologia de Alimentos

c/ Jaime Roig, 11  
Valencia—10

*Spain*

#### **Indian Standards Institution**

The following standards have been published:

- IS: 6850-1973 Agar, Microbiological Grade, Rs 2.50
- IS: 3044-1973 Mutton and Goat Meat, Curried and Canned, Rs 4.00

### **Minutes of the Meeting of the Bangalore Chapter held on 21st May 1974.**

Shri Panduranga Setty chaired the meeting and introduced the guest speaker, Dr B. S. Ramappa, Chief Scientific Officer, University of Agricultural Sciences, Bangalore.

Introducing the subject Dr Ramappa said that Poultry industry was a nascent one as it was born only about two decades ago. Before that it was a cottage industry in which a few birds were kept in the backyard. Because the birds did not get sufficient care, the production was highly uneconomical. To overcome the defects in such a management, interest developed in evolving scientific methods of poultry keeping to increase the production of eggs and meat. In this attempt the considerations were for developing new breeds, providing a balanced ration, good house keeping and management, preventive measures to overcome diseases and overall sanitation. In addition, there was need for excellent marketing and obtaining financial backing.

The speaker also mentioned that there was need for consumer education in the marketing of the poultry products. Poultry is now providing a means of improving the nutrition of the average Indian, by making available high class proteins and vitamins. Eggs provide an excellent food for children and adults alike. The industry is now being financed by cooperatives and nationalised banks. He then described some of the non-edible uses of egg albumin. The talk was followed by an interesting discussion.

### **Minutes of the Meeting of the Bangalore Chapter held on 18th June 1974.**

Shri Panduranga Setty was in the chair and Dr B. L. Amla, Director, Central Food Technological Research Institute, Mysore addressed the gathering.

Shri Panduranga Setty introducing Dr Amla said that he was a very familiar figure to all the food scientists and food technologists in the country and abroad, as he had contributed considerably to the progress of Food Science. Dr Amla then in his talk on 'Challenging Tasks for Food Scientists and Technologists' said that food technology was evolved over the past centuries from the time man took up agriculture. In India over 70 per cent of the popu-

lation live in villages and produce food for the entire nation. In America only 6 per cent were directly connected with agricultural production, and in USSR it is only 12 per cent. In the near future, with the present industrialisation of the country, India may also face a situation when a small agricultural population will have to produce all the commodities for the entire nation. Problems of storage, waste prevention, conservation and transport will get magnified. It will be the effort of the food scientists and technologists that would contribute to build links between the agriculturist and the urban consumer. Marketing thus becomes an important aspect. Grain is staple food and constitutes about 80 per cent of the diet in India. About 70 per cent of the grain grown in the country is retained by the villagers for their consumption. This grain is highly susceptible to damage and deterioration because of poor conditions of storage and handling. The magnitude of this loss becomes apparent even if we assume that the loss is not more than 10 per cent. Of the 26 million tons of wheat produced only 10 per cent goes through the organised milling industry. We do not have a correct knowledge of the loss of the nutritional quality of the wheat flour which is processed through the village *chakkis*.

The annual production of fruit and vegetable in the country is of the order 20 to 30 million tons per annum. This is only 1/4 of the requirement of the country. Only about 3 to 4 per cent of the fruit grown in the country goes into the processing units. In Europe and America the growing season of fruits is limited to certain months. But in India because of its tropical and sub-tropical climate some fruits are available throughout the year. Hence the dietary habits of the people is such that they demand fresh fruits without going for canned fruits. Nearly 80 per cent of the canned fruit produced is sold to the Services. Western housewife relishes cooked fruits; but such foods are not popular in our country. Though convenience foods are still in the beginning of their entry into the market, they have good value because the housewife does not mind paying a little extra for the convenience they provide.

The Food Technologist in India has the formidable task of catering to different tastes. The food habits of people in Kashmir or in Punjab are not the same as in Tamil Nadu or in Kerala. It is not

possible to fix the profiles for the country as a whole, and hence it is to be done on regional basis. In addition he has to work with a social system which has incorporated in it many taboos.

Dr Amla concluding said there were a number of challenges which the Food Scientists have to take up and solve. The problems are everywhere, the solutions are urgently needed and it is in this milieu that the technologists have to function in India. Mr M. R. Chandrasekhara proposed a vote of thanks at the end.

### New Members

- Mr S. K. Khanwalker, C/o B. M. Khanwalker, Principal, Govt. Inter College, *Allahabad-211001*.
- Mr V. N. Madhavan, 143, Sindhi Society, Chembur, *Bombay-400071*.
- Mr H. R. Adhikari, Biochemistry and Food Tech. Divn., Bhabha Atomic Research Centre, Trombay, *Bombay-400085*.
- Mr V. Sudha Rao, Bhabha Atomic Research Centre, Trombay, *Bombay-400085*.
- Mr S. B. K. Warriar, Biochemistry and Food Tech. Divn., Modular Laboratories, Bhabha Atomic Research Centre, Trombay, *Bombay-400085*.
- Mr Nerkar Devdas P., Scientific Officer, Biochemistry and Food Tech. Divn., Bhabha Atomic Research Centre, *Bombay-400085*.
- Mr D. R. Bongirwar, L/2 Atomic Energy Quarters, 'Mandala', Sion-Trombay Road, P.O. Deonar, *Bombay-400088*.
- Dr Paul Thomas, Biochemistry and Food Tech. Divn., Bhabha Atomic Research Centre, *Bombay-400085*.
- Mr K. G. Pinge, D85/879, M. I. G. Colony, Gandhinagar, Bandra (East), *Bombay-400051*.
- Mr K. M. Agashe, Chemistry Department, Victoria Jubilee Technical Instt., Matunga, *Bombay-400019*.
- Mr A. N. Roy, Biochemistry and Food Tech. Divn., Modular Laboratory, Bhabha Atomic Research Centre, Trombay, *Bombay-400085*.
- Mr Sheo Ram Agarwal, E4/12 Sunder Nagar, S. V. Road, Malad (W), *Bombay-400064*.
- Mr A. J. Shrikhande, Biochemistry and Food Tech. Divn., Bhabha Atomic Research Centre, Trombay, *Bombay-400085*.
- Mr A. J. Chandak, C/o Shri J. M. Chandak, Amaravati Camp, Amaravati, Maharashtra.
- Mr Navin Shah, Bahuadaraj Road, Purshottam Estate, Baroda.
- Mr R. D. Patel, Post Box 40, National Dairy Development Board, Anand, *Gujarat*.
- Mr V. M. Dhekme, 17A, Bhaguagar Colony, Dharampett, *Nagpur*.
- Dr O. P. Agarwala, University of Washington-Fisheries, Seattle, Washington 98195, *U.S.A.*
- Mr O. P. Powar, Kumud Kunj, Dr Ambedkar Road, Mulund West, *Bombay*, Maharashtra.
- Mr K. D. Powar, Kumud Kunj, Dr Ambedkar Road, Mulund West, *Bombay*, Maharashtra.
- Mr J. C. Kathrani, 126, Princess Street, Asian Chemical Works, *Bombay-400002*.
- Mr Hari Charan Lal, James Smith and Co., Rallie India Limited, 21-Raveline Street, *Bombay-400001*.
- Mr Brijesh Narain, 127, New University Hostel, Matunga, *Bombay-400019*.
- Mr Sudha K. Asundi, 12 Om Shanti Main Avenue, Santa Cruz (W), *Bombay-400054*.
- Mr Divakar Rao, A 63, DII Flats, Moti Bagh, *New Delhi-110021*.
- Mr S. D. Dharkar, Tata Oil Mills Co. Ltd., Loni Road, Mohan Nagar P.O. *Ghaziabad*, U.P.
- Mr M. Satyanarayana, Tata Oil Mills Co. Ltd., Loni Road, P.O. Mohan Nagar, *Ghaziabad*, (U.P.)
- Mr H. S. Ramaswamy, No. 21, 5th Cross, Malleswaram, *Bangalore-560003*.
- Mr M. S. Mohan, Defence Food Research Laboratory, *Mysore-570010*.
- Mr K. R. Viswanathan, Defence Food Research Laboratory, *Mysore-570010*.
- Miss D. Rajalakshmi, Industrial Research, Consultancy and Extension, C.F.T.R.I., *Mysore-570013*.
- Miss H. N. Malathi, Industrial Research, Consultancy and Extension, C.F.T.R.I., *Mysore-570013*.
- Mr Vinod G. Sarob, No. 1294, Housing Board (Opp. Circle Telegraph Stores), Magadi Road, *Bangalore-40*.
- Dr N. Kirpalani, E-23, Defence Colony, *New Delhi-24*.
- Mr Govindan Velaydhan Nair, Indexport, Hindustan Lever Limited, Backbay Reclamation, 165/166, *Bombay*, 400020.
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- Mr K. Gopinathan Nair, C.F.T.R.I., *Mysore-570013*.

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- Mr Jhaveri Prakash, C/o Prashant Corporation, 1511, Prasad Chambers, Bombay-400004.
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- Mr Vrajlal A. Sura, Gujarat Cold Storage, Harganga Mahal, Khodadad Circle, Dadar, Bombay-400014.
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- Mr S. Venkatanarayana, Microbiology Discipline, CFTRI, Mysore-570013.
- Mr C. V. Narayanan, Packaging Technology, CFTRI, Mysore-570013.
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- Mr S. Anandaraman, PP and FT Discipline, CFTRI, Mysore-570013.
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# SYMPOSIUM ON FISH PROCESSING INDUSTRY IN INDIA

ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS (INDIA)

Central Food Technological Research Institute, Mysore - 570 013

Association of Food Scientists and Technologists (India) takes pleasure in announcing a Symposium on '*Fish Processing Industry in India*' to be held in CFTRI, Mysore, in the first week of February 1975. The main objectives of the Symposium are :

- i. To provide a common platform for the industry connected with fish processing and those who are involved in the R and D activities in the area.
- ii. To focus attention on the problems in the marketing and export of processed fish products.
- iii. To discuss and identify areas for future research and development activities in :
  - (a) Raw-material resources—availability and potentialities.
  - (b) Post-harvest handling, distribution for fresh consumption, use of ice, containers, transport, bacteriology and public health aspects.
  - (c) Freezing of fish for export and internal consumption, prospects and necessity of cold chain.
  - (d) Canning.
  - (e) Traditional cured fish products, its future and scope.
  - (f) Development of new products such as fish sausage, fish hydrolysate, fish protein concentrate and other dehydrated fish products.
  - (g) Development of technology for utilization of crustaceans and molluscs.
  - (h) By-products such as fish meal, fish oil, etc.
- iv. Machinery and equipment for fish processing industry—indigenous availability and needs.
- v. Quality control, standards and specifications.

It is intended to invite lead papers from eminent specialists on various subjects along with papers from research workers in the field. An exhibition of various fish products and machinery involved in fish processing will be another feature of the Symposium. Those who are interested in participating in above Symposium may kindly contact the Hon. Exec. Secretary for further particulars.

DR. T. N. RAMACHANDRA RAO  
*President*

DR. V. H. POTTY  
*Hon. Exec. Secretary*