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RESEARCH PAPERS

Antinutritional Factors of *Phaseolus mungoreous* (*Phaseolus mungo* var. M_{1-1} X *Phaseolus aureus* var. T_1)

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The total flatulence factor in *Phaseolus mungoreous*, obtained by crossing *P. mungo* var M_{1-1} with *P. aureus* var T_1 was lowest (26.9 mg/g of flour) while it was highest in M_{1-1} (31.0 mg/g flour). The trypsin inhibitor activity was also low (8.72 to 82.73 units/mg of protein) in the hybrid as compared to the parents. Heating (5 min) destroyed completely the trypsin inhibitor but the Hemagglutinin activity was destroyed upto 95.6% in 5 min heating and 99.2% in 10 min heating.

Pulses contain high amounts of protein and carbohydrate but low level of oil[1]. Despite the high protein content their maximum contribution to nutrition has not been fully exploited in many parts of the world because of the problem of antinutritional factors, such as trypsin inhibitors; low level of sulphur amino acids, particularly methionine.[2] Hemagglutinin in legume seeds has long been recognised. A number of proteins with trypsin inhibitory activity in kidney beans at various stages of germination has been reported.[3]

Ikegwnonu and Bassir[4] reported that the administration of phytohemagglutinins (Lectins) to rats induced impairment of liver functions, activation of some dehydrogenase enzymes connected with carbohydrate metabolism and coagulative necrosis of the liver. They also reported the specific hemagglutinating activities in soybean and *Phaseolus lunatus*.

Legumes contain a considerable amount of oligosaccharides which have been implicated as a factor responsible for flatulence[5,6]. It will, however, be of much advantage to produce varieties in which the protein level is raised and flatus factors, trypsin inhibitors and phytohemagglutinin are reduced. However, it may be beneficial to produce varieties with a higher protein content and lower levels of antinutritional factors. In such an attempt, a variety, *Phaseolus mungoreous* a cross between *Phaseolus mungo* (M_{1-1}) *Phaseolus aureus* (T_1) was developed its nutritional quality is reported.

Material and Methods

Samples of the cross between *mash* (*Phaseolus mungo*) var. M_{1-1} and *moong* (*Phaseolus aureus*) var-T_1 which is called as *Pheseolus mungoreous* (amphidiploid) and the parents were obtained from the Department of Genetics, Haryana Agricultural University.

Preparation of samples: Different samples were thoroughly cleaned free of any dirt or foreign matter. Seeds were graded and bold seeds were kept for germination study. Rest of the samples of each variety was ground to pass through 100 mesh sieve and stored in air tight containers until used for analysis.

The samples were analysed for soluble carbohydrates, oligosaccharides, trypsin inhibitor activity and haemagglutinins. The effect of germination and heating on the trypsin inhibitor activity was also studied.

Soluble carbohydrates and flatus factors: Total water soluble carbohydrates, and reducing sugars were determined by potassium ferricyanide reduction method[7]. Clarification of the hydrolysate was done according to AOAC method[8] and the extract obtained was hydrolysed by the method of Srinivasan and Bhatia[9].

Quantitative determination of the oligosaccharides, sucrose, raffinose and stachyose was carried out by the method of partition paper chromatography, using ethyl alcohol: acetic acid: formic acid: water (9:1.5:5:2) solvent by the descending solvent technique of Bailey and Pridham[10]. The sugar in the eluate extract after chromatographic run was determined by the phenol-sulphuric acid method[11].

Trypsin inhibitor activity: Seeds were treated with 0.05 N mercuric chloride for 3 to 5 min and then washed several times with sterile distilled water. Seeds were then soaked in sterile distilled water for 2 hr. Seeds were germinated in petridish lined with two folds of filter paper (Whatman No. 1) and kept in an incubator at 35°C±0.5. Seedlings were moistened twice a day with sterile water.

Germination counts were taken after 12 and 18 hr. Samples were taken for analysis at definite intervals. The effect of heating on the TI activity of raw samples was tested by heating them with 10 ml of water in a boiling water bath for different intervals.

Trypsin inhibitor (TI) extract was prepared[12] and trypsin inhibitor activity (TIA) was determined accor-

TABLE 1. SOLUBLE SUGARS AND FLATULENCE FACTORS IN *Mash* AND *Moong* VARIETIES AND THEIR CROSS

Variety	Soluble sugars			Flatulence factors			Total flatus factors (%)
	Total (%)	Reducing (%)	Non-reducing (%)	Sucrose (%)	Raffinose (%)	Stachyose (%)	
Mash M_{1-1}	7.20	1.00	6.20	1.06	0.87	1.17	3.10
Moong T_1	6.25	2.45	3.80	0.83	0.73	1.16	2.72
Amphidiploid (cross)	9.13	1.86	7.27	0.56	1.03	1.10	2.69

ding to the method described[13]. The deproteinised hydrolysate was used for the colorimetric determination of tyrosine with phenol reagent[14] and protein content in TI extract was estimated by the method of Lowry *et al*[15].

The trypsin inhibitor activity was expressed as trypsin units inhibited, one trypsin unit being defined arbitrarily, the amount of enzyme that liberates one micro mole of tyrosine under given assay conditions.

Hemagglutinating activity: Hemagglutinin activity was estimated[16] and expressed as HU (Hemagglutinin units) and the effect of heating was also tested.

Results and Discussion

Total carbohydrates, reducing sugars and non-reducing sugars: The data presented in Table 1 reveal that the values for reducing sugars are lowest in M_{1-1} (10.0 mg/g flour) and highest in T_1 (24.5 mg/g flour), while in the cross (amphidiploid), it is 18.6 mg/g flour. Non-reducing sugars are highest in amphidiploid (cross) (72.7 mg/g flour) and lowest in T_1 variety (38.0 mg/g flour). Amphidiploid also has the highest amount of total sugars (91.3 mg/g flour) in contrast to its parents in which it ranged between 62.5 and 72.0 mg/g flour.

Flatus factors: Phaseolus aureus (T_1), *Phaseolus mungo* (M_{1-1}) and their cross *phaseolus mungoreous* (amphidiploid) were analysed for their flatulence factor contents. The results obtained are presented in Table 1. All the pulses have fairly high oligosaccharide content.

Total flatulence factors ranged from 26.9 to 31.0 mg/g flour. Sucrose content is lowest in the cross (*Phaseolus mungoreous*(0.56 percent) and highest in mash variety M_{1-1} (1.06 percent). It is concluded from the data that the cross contained the least amount of stachyose (1.1 percent) as compared to the parents, though the different pulses did not show marked variations in their content. The results would indicate that all the pulse groups had fairly high content of flatulence factors with *mash* showing the highest content, with the cross containing the least.

Murphy[17] obtained a sample reportedly gasless member of *Phaseolus vulgaris*, Pike's Jacobs Cattle bean, which was the result of a cross of a reportedly gasless heirloom Jacobs Cattle bean (*Phaseolus vulgaris*) with the black Mexican variety. The significance of these limited flatulence measurements on the members of the dry bean family is that there appears to be a genetic transfer of the degree of flatulence response. This suggests a possibility of reducing this undesirable legume trait by genetic selection.

Trypsin inhibitors and effect of germination and heating: The changes in trypsin inhibitor activity and extractable protein in raw seeds and during germination was studied and the data are presented in Table 2 and 3. It is evident from the data (Table 2) that there is complete germination in *moong* after 12 hr, while in amphidiploid and *mash* it is only 50 55 percent respectively. After 18 hr, there was hundred per cent germination in all the varieties.

From the data in Table 3. it is seen that trypsin inhibitor at zero time, which is the content in raw seeds, is the highest in *moong* (12.16 units/mg protein) and lowest in the cross (8.72 units/mg protein). The protein content is the highest in the cross (21.48 mg/ml extract) as compared to that of its parents, which ranged from 15.85 (T_1) to 17.83 (M_{1-1}) mg/ml of the extract.

Trypsin inhibitor content of 11.81 in the dormant seeds rose to 91.43 (units/mg protein) after 84 hr of germination in *mash* M_{1-1} variety, from 12.16 to 117.63 units/mg protein in moong T_1 variety, and from 8.72 to 82.73 units/mg protein in amphidiploid. Amphidiploid contained lowest amounts of trypsin inhibitor in the dormant seeds as well as after 84 hr of germination as compared to its parents.

TABLE 2. PERCENTAGE GERMINATION OF *Mash* AND *Moong* VARIETIES AND THEIR CROSS

Variety	Period of germination	
	12 hr	18 hr
Mash M_{1-1}	55	100
Moong T_1	100	100
Amphidiploid (Cross)	50	100

TABLE 3. EXTRACTABLE PROTEIN AND TRYPSIN INHIBITORS DURING GERMINATION IN DIFFERENT VARIETIES OF *Mash*, *Moong* AND THEIR CROSS

Time (hr)	*Phaseolus mungo* (M_{1-1})			*Phaseolus aureus* (T_1)			*Phaseolus mungoreous*		
	TIA/ml extract	Protein (mg/ml extract)	Sp activity (units/mg protein)	TIA/ml extract	Protein (mg/ml extract)	Sp activity (units/mg protein)	TIA/ml extract	Protein (mg/ml extract)	Sp activity (units/mg protein)
0	210.6	17.83	11.81	192.8	15.85	12.16	187.4	21.48	8.72
12	244.2	13.37	18.26	241.2	9.23	26.13	244.2	13.81	17.64
24	56.6	9.17	6.17	62.6	5.38	11.63	83.8	8.91	9.40
36	237.4	10.45	22.71	225.0	8.79	25.59	230.4	13.37	17.23
48	141.0	5.74	24.35	112.4	3.52	31.64	176.8	5.79	21.89
72	294.0	4.78	57.02	266.2	2.35	113.27	289.2	4.70	61.53
84	260.6	2.85	91.43	248.2	2.11	117.63	248.2	3.00	82.73

The lowest trypsin inhibitor content was observed after 24 hr of germination in all the varieties of pulses, but the cross had a little higher content as compared to its dormant seeds after 24 hr of germination.

Protein content declines slowly with the progress of germination in all the varieties. Protein content (mg/ml extract) ranged between 17,83 and 2.85 in *mash*, 15.85 and 2.11 in *moong* and 21.48 and 3.00 in amphidiploid during 84 hr of germination interval, although there was a slight increase in protein content after 36 hr of germination. With the onset of germination, however, the cells of the seed changed to a state of vigorus metabolic activity. The results obtained suggest that the main function during germination of these protein components might not be related to their trypsin inhibitor activity.

Trypsin inhibitor activity increased slowly after 30 hr of germination in all the varieties of pulses. A marked difference in TIU/mg of protein was observed in all the varieties during germination.

The effect of temperature (heating) on trypsin inhibitor of all the varieties was studied. Heating for 5 min resulted in the total loss of trypsin inhibitor in all the pulse flours. The extent to which the trypsin inhibitor in legume is destroyed by heat is a function of temperature and the duration of heating.

TABLE 4. EFFECT OF HEAT ON PHYTOHEMAGGLUTINATING ACTIVITY IN SEEDS OF *Mash* AND *Moong* AND THEIR CROSS

Variety	Hemagglutinin units (H.U.)		
	Raw seeds	After 5 min	After 10 min
Mash M_{1-1}	800	42.6	20
Moong T_1	800	37.3	16
Amphidiploid (Cross)	800	37.3	8

An improvement in nutritive value does accompany the inactivation of trypsin inhibitors by heat treatment. Germination is known to result in an improvement in the nutritive value of a number of legumes. That effect appears to be unrelated to any change in trypsin inhibitor activity in the germinated seeds[18]. These protease inhibitors have attracted the attention of nutritionists because of the possible role they might play in determining the nutritive value of plant proteins.

Phytohemagglutinin: Phytohemagglutinating activity in raw seeds and the effect of heating is given in Table 4. Hemagglutinating activity in the parents and hybrid was found to be 800 HU (Hemagglutinating Units) in raw seed floor. Effect of heating was significant on hemagglutinating activity in these varieties. When the pulse extract was heated for 5 min, 94.6 to 95.4 percent of hemagglutinin was destroyed. Heating the extract for 10 min destroyed 97.5 percent activity in *mash*, 98 percent in *moong* and 99.2 percent in the cross. The significance of hemagglutinins in human nutrition has not yet been established. Thus, it is important that continuing efforts be made to breed legumes (particularly *Phaseolus*) which have low hemagglutinating activity.

It must be emphasised that the first order of priority in approaching these objectives will be the improvement of productivity, adaptability and yield stability. The second is improving the acceptability and the food value of legumes as carriers of nutrients and reducing the concentration of certain undesirable constituents.

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Sugars, Organic Acids and Amino Acids in *Anab-E-Shahi* Grape During Growth and Development

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Anab-E-Shahi **grape cultivar took 90 days from anthesis to ripening. The berry growth gradually increased upto 40 days after anthesis, followed by sharp increase upto 80 days and declined there after. The berry density which was low initially increased gradually till ripening. Very little starch was noticed in berry and it showed a decreasing trend upto ripening. Total sugars, glucose and fructose contents increased with ripening. Sucrose was not initially detected. Non-volatile acidity formed 50-80 per cent of the total titratable acidity. Tartaric: malic, and citric acids were identified in all developmental stages. During ripening malic acid content decreased. But tartaric: malic acid ratio remained constant. Eighteen amino acids were identified. Their concentrations changed markedly from anthesis to ripening.**

The growth and development of grape berries have been the subject of a number of studies[1-3]. Investigations on *Bangalore Blue* grapes was reported earlier[4]. *Anab-E-Shahi*, a commercially important grape variety, occupies a pre-eminent position as a table variety in the southern part of this country. Systematic growth studies, coupled with changes in contents of important bio-chemical constituents, could lead us to better understanding of the processes associated with ripening and enables in harvesting quality fruits at appropriate time. The present paper is therefore, concerned with the changes in sugars, non-volatile organic acids and soluble amino acids associated with ripening of *Anab-E-Shahi*, grapes.

Materials and Methods

The procedure adopted in selecting and sampling of grape bunches was same as reported earlier[5]. Twenty berries collected at random from a number of panicles were used for calculating the average length, diameter, volume and weight of single berry. Juice extracted from berry by crushing and squeezing through two layers of muslin cloth was used for measuring total soluble solids by hand refractometer. For estimation of acidity, berries were blended with distilled water and the filtered water extract was titrated against standard alkali. Dry matter, alcohol insoluble solids and starch were estimated by standard AOAC methods[6]. A combination of cationic and anionic chromatography was used to purify amino acids, organic acids and sugars. Known quantity of berry was extracted in a Soxhlet extractor with 80 per cent ethanol. The extract after removing ethanol was passed through Dowex 50×8 (H^+ form) and Amberlite IR-4B (OH^- form) resin columns respectively. The filtrate after passing through the two columns was taken for sugar analysis. The method employed for fractionation and estimation of amino acids, organic acids and sugars were the same as reported earlier[7].

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TABLE 1. DEVELOPMENTAL PATTERN OF *Anab-E-Shahi* GRAPE BERRY

Days after anthesis	Av length of berry (cm)	Av diam of berry (cm)	Av wt. of berry (g)	Av vol. of berry (ml)	Fruit density	Rate of berry growth	
						mg/day	ml/day
15	0.92	0.64	0.25	0.30	0.833		
30	1.68	0.98	0.90	1.00	0.900	43.3	0.047
45	2.14	1.29	1.82	1.90	0.957	61.3	0.060
60	2.58	1.53	3.11	3.08	1.009	86.0	0.077
70	2.75	1.80	4.90	4.70	1.042	179.0	0.162
80	2.92	2.01	6.65	6.30	1.055	176.0	0.160
90	2.98	2.01	6.90	6.60	1.043	25.0	0.030
100	3.00	2.00	6.93	6.64	1.045	3.0	0.004

Results and Discussion

Anab-E-Shahi grape took 90 days between anthesis and ripeness. During this interval, volume, weight, length, diameter and density of berry varied considerably (Table 1). Berry development in terms of increase in length, diameter, volume and weight was rapid upto 80 days and then declined. The rate of berry growth as measured by the increase in volume and weight per unit time registered a gradual increase upto 45 days followed by a sharp increase upto 80 days and declined thereafter. The berry density was minimum at initial stage and increased gradually till ripening. Such increase in density was ascribed to the accumulation of organic acids initially and then to glucose and fructose[8].

The data on changes in sugars and acids at different developmental stages are given in Table 2. Percent dry matter of berry decreased upto 30 days, thereafter it increased. The increase in concentration of solutes, especially due to accumulation of acids and sugars whose concentration rose severalfold at the latter developmental stages, could account for such an increase in dry matter. Alcohol insoluble solids content registered a progressive decrease upto 80 days followed by slight increase at ripe and overripe stages. Starch is not found to accumulate in greater amounts in grape berry due to its non-climacteric nature[9]. In the present study also very little starch was noticed and it was 0.67 and 0.12 per cent at initial and ripe stages respectively. Similar results were reported from studies involving other grape varieties[10]. The decrease in starch concentration from early to ripe stage could be either due to increased activity of starch hydrolysing enzymes or to its dilution with berry size increase. At anthesis most cells of pericarp of berry contained starch granules which disappeared as the berry grew and at maturity they were reported to be present only in the epidermis[11].

The percentage of sugars namely, glucose and fructose was low till 45 days following anthesis and increased several-fold during later developmental stages. Its increase was ten and twenty-fold, from 45 to 60 and 45 to 90 days respectively. The level of glucose was higher than that of fructose upto 70 days, thereafter the reverse trend followed. Sharp increase in fructose concentration and decrease in glucose concentration was reported to be associated with senescence[12]. Sucrose was not detected upto 60th day. Later it was found to be present only

TABLE 2. SUGARS AND ACIDS IN DEVELOPING *Anab-E-Shahi* GRAPE BERRY

Days after anthesis	Dry matter (%)	Alcohol insolubles (%)	Starch (%)	T.S.S.	Sucrose (%)	Glucose (%)	Fructose (%)	Total acidity*	Sugar-acid ratio
15	9.82	5.09	0.67	—	**	0.12	0.13	2.61	9.10
30	6.95	2.68	0.66	—	**	0.15	0.10	3.26	0.07
45	7.64	2.73	0.60	—	**	0.36	0.11	2.06	0.22
60	10.71	1.74	0.48	10.1	**	2.23	1.97	1.56	2.69
70	15.34	1.59	0.36	11.1	0.07	2.91	2.87	0.91	6.44
80	16.63	1.48	0.32	14.6	0.08	3.86	4.13	0.74	10.91
90	16.73	1.92	0.12	15.4	0.11	4.14	5.18	0.69	13.66
100	17.68	2.25	0.11	16.8	0.27	3.99	5.56	0.57	17.24

*g of Tartaric acid/100 g. ** Not present.

in low amounts i.e. from 0.07 to 0.27 per cent. The predominance of glucose and fructose and the absence of appreciable amounts of sucrose was ascribed to the hydrolysis of the latter[12]. The glucose-fructose ratio was found to increase upto 45 days and afterwards decreased. Preferential utilisation of glucose over fructose by the dividing cells in earlier stages of growth of berry could account for such increased ratios. A similar trend in glucose-fructose ratios was reported by Kliwer[13]. He has suggested that the glucose-fructose ratio should be between 0.7 and 0.97 for table grape varieties at ripe stage. *Anab-E-Shahi* grape recorded a ratio of 0.80 at ripe stage which conformed to the standard prescribed for table variety.

Titratable acidity, a measure of all neutralisable acidic groups showed the familiar pattern of increase upto 30 days followed by a gradual decrease till ripening. The observed increase in acidity could be the result of accumulation of organic acids on account of translocation from leaves or synthesis within berry itself[8]. Sugar-acid ratio first declined upto 30 days and afterwards increased till ripening. The same increasing trend was observed with T.S.S.-acid ratio. It is generally agreed that the sugar-acid and T.S.S.-acid ratios are useful criteria for fixing the harvesting index of fruits. In *Anab-E-Shahi* grape, at ripe stage, the values recorded were 13.66 and 22.31 for sugar-acid and T.S.S.-acid ratios respectively.

Non-volatile organic acids which formed 50-80 per cent of the titratable acidity showed a decreasing trend from anthesis to ripening. Tartaric and malic acids were the major acids. Small quantity of citric acid was also observed. The level of tartaric acid was highest in the initial stage and that of malic acid 70 days after anthesis. Tartaric acid concentration fell steadily till sensecence. The concentration of malic acid fell during ripening and as maturity approached, tartaric and malic acid concentration remained fairly constant. The tartaric-malic acid ratio which decreased considerably from the initial stage to 70 days remained more or less constant as maturity approached. Citric acid concentration increased upto 60 days and decreased gradually thereafter.

The ripening period in *Anab-E-Shahi* grape was found to be associated with a sharp decrease in malic acid concentration. The observed increase in tartaric-malic acid ratio could be explained by this decrease. The decrease in malic acid concentration could be attributed to its increased utilisation in respiration or reduction in the amount translocated from leaves[14]. The reduction could also be due to loss in the activity of CO_2 fixing mechanism or the loss of enzymes necessary for reactions intermediate to malate synthesis[14]. It may also be possible that malic acid and other organic acids are used for synthesis of carbohydrates[9]. The observed increase in Sugar-acid ratio during ripening indicates such possibility. The preferential utilisation of organic acids in respiration processes could be another reason. Studies involving ^{14}C malic acid has indicated the increased metabolism of malic acid to CO_2 with the ripening of *Vitis vinifera* grapes[15]. Most of the organic acids appeared to have been oxidised completely during ripening[16]. The decrease in acidity observed during ripening could be the result of oxidation of acids.

Eighteen amino acids tentatively identified as alanine, α-aminobutyric acid, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan and valine were found to be present in varying concentrations at different developmental stages. The soluble amino acids concentration increased upto 80 days, decreased at ripe stage and again started increasing at senescence. Kliewer[17] reported 2 to 5 fold increase in total soluble amino acids during ripening of grape berries. The observed increase could be attributed to the result of lessened demand for these metabolites as growth processes are slowly taken over by ripening processes in the latter stages of development[17].

The concentration of individual amino acids changed markedly from anthesis to ripening. Glutamic acid was the predominant amino acid present during initial stage followed by aspartic acid, proline, lysine, methionine, glycine, serine, leucine, histidine and alanine. However, at ripe stage, arginine recorded the highest concentration followed by alanine, proline, serine, aspartic acid, lysine and valine. The concentration of alanine, arginine and proline increased several folds during the latter developmental stages. The arginine content increased rapidly upto ripening and decreased at and after maturity, whereas proline increased during maturity and senescence stages. Arginine-proline ratio was low in initial stage, registered sudden increase after 45 days to 80 days and then decreased. The large increase in proline concentration observed could be related to senescence[17]. The changes observed in proline content during different developmental stages of *Anab-E-Shahi* grape were in general agreement with the reported findings[18]. The low arginine content could be the characteristic of growth phase and its increased concentration could be the characteristic of ripening process. Such conclusions were drawn from the study on *Thompson Seedless* grape also[17].

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Dehydration of Green Chillies *(Capsicum frutescens)*

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Using a cabinet drier, standard conditions and pretreatments required to produce the best quality dehydrated green chillies were determined. The product so obtained was subjected to storage for 60 days, the equilibrium relative humidity determined and the product was evaluated subjectively as well as with respect to chlorophyll retention and changes in rehydration ratio.

In India, many varieties of green chillies are available throughout the year some being seasonal. They are used in food preparation in raw state. In the present investigation an attempt was made to dehydrate fresh green chillies with the minimum damage to the desirable attributes.

The experiments were carried out with the Pune variety (*Capsicum frutescens* var. *accuminata Fingerh*). of chillies. Different pretreatments were given to the raw chillies prior to dehydration in a cabinet drier at 60 ± 1°C and conditions as shown in Table 1 were used. The drying in cabinet drier was with air of relative humidity of 60 per cent and velocity of 700 ft/min. The tray load was 3.7 kg/sq.m. Drying at temperatures of 70 ± 1° and 80 ± 1°C was also carried out to a moisture level of 5 per cent from initial moisture content of 83-86 per cent in fresh green chillies, after giving the standard pricking, blanching and lye treatment as found best for drying at 60°C. The best product obtained from the above experiments was stored for 2 months in light in unsealed polyethylene bags (200 gauge), at 25° and 37°C and at five different relative humidities (20, 40, 60, 80 and 90 per cent) and evaluated subjectively and also analysed for chlorophyll[1], capsaicin[2,3], rehydration ratio-after complete drainage of water[4], dehydration ratio[4], and moisture content[5].

A batch of 500 g of green chillies was dried at 60°C after different pretreatments as shown in Table 1 and product evaluated both in the dried and rehydrated conditions. The criteria used for evaluation were chlorophyll retention, turgidity, flavour, colour, appearance and moisture content. Pricking and blanching treatment is known to increase[6] the rate of drying and this is confirmed in the present study also (Table 1). Treatments of unblanched chillies with 2 per cent sodium carbonate showed almost 91.1 per cent chlorophyll retention, but the samples had uneven green patches with yellowish green appearance. Samples blanched and treated with 2 per cent sodium carbonate had poorer retention of chlorophyll and the SO_2 treatment resulted in poor retention of chlorophyll in both unblanched and blanched samples. Chillies pricked, blanched and treated with 2 per cent sodium hydroxide for 10-20 min. and washed from excess alkali gave the best product when dried

TABLE 1. EFFECT OF PRETREATMENTS ON QUALITY OF DEHYDRATED* CHILLIES

Treatments	Moisture (%)	Drying time (Hr.)	Dehydration ratio	Rehydration ratio	%mg. Chlrophyll	Quality of dehydrated product	Quality of Rehydrated Product
1. Unblanched	5.5	12	8	6.9	62.6	Yellowish green, opaque, Burnt, less pungent flavour.	Palegreen, wrinkled and flabby, Fresh capsicum odour lacking.
2. Unblached and pricked	5.5	10	8	7.0	63.0	– do –	– do –
3. Pricked, unblanched +2% Na_2CO_3[a]	4.4	10.5	8.3	6.85	65.1	Yellowish green patches, less pungent flavour.	Yellowish green with uneven green patches, less intense pungent, aroma.
4. Pricked, unblanched +2000 ppm SO_2[b]	9.0	10	8.5	6.3	43.2	Yellowish green, less intense flavour.	Less green, flabby, weakly pungent.
5. Pricked and blanched[c]	6.1	10	7.7	6.9	51.2	Brownish green translucent, mildly pungent, cooked flavour.	Highly flabby, brownish slightly wrinkled.
6. Pricked, blanched +2% Na_2CO_3[a]	4.1	10	8.3	7.1	53.2	Wrinkled, brown with yellow patches, mildly pungent, cooked flavour.	Flabby, irregular brown and yellow patches, pungent flavour.
7. Pricked, blanched +2% NaOH[d]	5.5	10	8.3	7.2	65.3	Green, Opaque, cooked pungent flavour.	Green attractive with some turgidity pungent odour.
8. Pricked, blanched +2000 ppm of SO_2[b]	5.8	10	8.3	6.7	53.2	Yellowish green, cooked pungent flavour.	Flabby, uneven yellow patches, weakly pungent

a) Treatment with 2% Na_2CO_3 for 60 mins.

b) Treatment with 2000 ppm SO_2 for 15 mins.

c) Blanched for 4 min. in boiling water bath.

d) Treatment with 2% NaOH for 10 min.

* Temperature of dehydration was 60°C.

Initial chlorophyll content of fresh chillies is 71.5 mg. % Dry weight basis (DWB)

TABLE 2. EFFECT OF DRYING CONDITIONS ON GREEN CHILLIES

Dehydration temp. (°C)	Pretreatment	Dehydration time (hr)	Final moisture %	Dehydration ratio	Rehydration ratio	%Chlorophyll retention △	%Capsaicin retention ○	Appearance	Flavour
60	Pricked, blanched and lye treated	10	5.5	8.3	7.2	91.1	92.6	Dark green, flat and crisp, with little wrinkles, no separation of seeds	Pungent, cooked chilli aroma
70	,, ,,	6.5	5.0	9.8	7.2	88.5	90.0	,, ,, ,,	,, ,,
80	,, ,,	4.0	5.0	9.75	5.9	60.2	75.0	Yellowish green, crisp, flat, dark patches with no separation of seeds	Pungent, cooked chilli aroma and burnt flavour.
70	—	7.75	5.0	9.8	6.8	62.6	66.5	Yellowish green, opaque, flat, no separation of seeds.	Pungent.

△ Initial chlorophyll content in fresh chillies on DWB 71.5 mg%

○ Initial capsaicin content in fresh chillies on DWB 276.0 mg%

TABLE 3. EVALUATION OF DEHYDRATED CHILLIES AFTER 60 DAYS OF STORAGE AT

25°C				37°C			
Relative humidity	Rehydration ratio	%Chlorophyll retention	Subjective evaluation	Relative humidity	Rehydration ratio	%Chlorophyll retention	Subjective evaluation
%				%			
20	5.6	81.5	Green, crisp with dried chilli aroma	20	5.7	68.1	Green, crisp with dried chilli aroma
40	5.2	78.0	„ „	40	5.3	61.7	„ „
60	4.1	69.9	Uniform green colour with some yellow patches, musty odour, less crisp	60	4.6	37.8	Green with irregular yellow patches, musty odour, less crisp.
80	4.0	59.9	Yellowish, musty flavour, flabby.	80	4.5	20.1	Yellowish brown, musty flavour, flabby.
90	4.1	51.6	Brownish yellow, no original chilli flavour, soft.	90	3.8	15.45	Brownish yellow, no original chilli flavour, soft.

in cabinet drier at 60°C. The product possessed characteristic green colour with almost 91 per cent chlorophyll retention. It possessed an attractive shiny appearance without much wrinking on the pods. The product had a high rehydration ratio of 7.2 and the pH of the chillies was changed to 7.6 in dehydrated product from an initial pH of 5.9 in fresh.

Using the best pretreatments of blanching and alkali treatment as found above, chillies were also dried at 70° and 80°C in a cabinet drier for comparative evaluation as shown in Table 2. The product obtained at 70°C was found to be the best. It required only 6.5 hr for drying as compared to 10 hr at 60°C and chlorophyll retention was as high as 88 per cent, while the appearance of the product remained more or less similar. The product dried at 80°C developed a burnt tinge with low chlorophyll retention. Capsaicin content also decreased with increase in the temperature of drying. Lye treated samples dried at 70°C were found to retain capsaicin better than the untreated one.

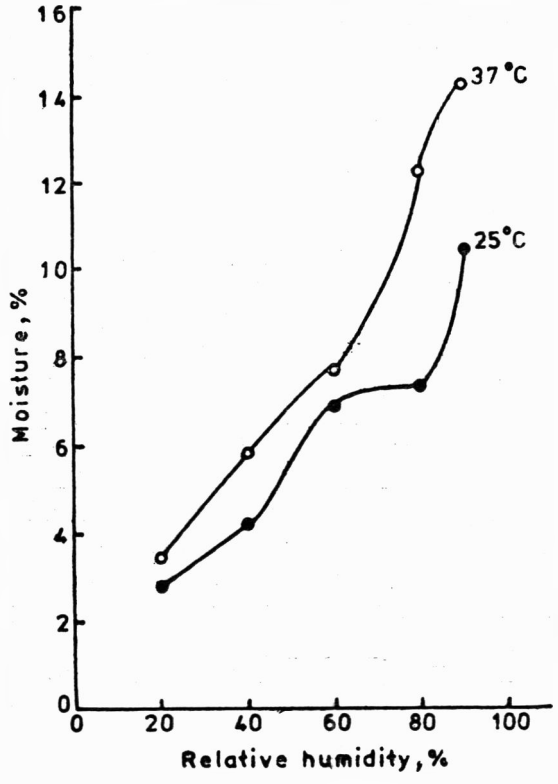

Fig. 1. Sorption isotherm for dehydrated green chillies

Chillies dried in cabinet drier at 70°C after pretreatments of pricking, blanching and 2 per cent lye treated were subjected to storage conditions at 25° and 37°C temperatures and relative humidities of 20, 40, 60, 80 and 90 per cent to assess their storage stability. From the sorption isotherm plotted in Fig. 1, the ERH of the dehydrated green chillies was found to lie between 40 and 60 as indicated by the inflexion point. Thus, the product when stored at this RH will be stable with respect to moisture loss or uptake. It was also observed (Table 3) that even after 60 days of storage at low RH (20-40 per cent) both at 25° and 37°C, the product retained 62-81 per cent chlorophyll as against samples stored at high RH. At 37°C degradation was greater and pods were yellowish brown with few patches of chlorophyll at relative humidities higher than 60 per cent. The rehydration ratio was almost the same at both the temperatures (25° and 37°C) of storage and was found to decrease with increase in RH in storage chamber, After 2 months of storage at 90 per cent RH, the rehydration ratio was found to be 4.1 and 3.8 at 25° and 37°C respectively. The higher rehydration ratio at high temperature may be due to the change in cellulose crystallinity[7] at high temperature. All these studies therefore revealed that the product can be best stored at 25°C and 40-60 per cent RH for at least a period of two months.

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Effect of Ripening of Cream, Manufacturing Temperature and Packaging Materials and Flavour and Keeping Quality of Ghee *(Butter fat)*

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Ripening of cream was without effect on the initial flavour of ghee (butter-fat) but enhanced its subsequent deterioration. The clarification temperature, which also controlled the contents of phospholipids, determined flavour and shelf-life of ghee, both being highest at 130°C. The keeping quality of ghee in polyethylene plastic containers was as good as in brown glass bottles. The free fatty acid development of ghee was closely related to its flavour quality, but thiobarbituric acid value, iodine number and carbonyl content were unrelated.

In India, ghee (butter fat) is traditionally packed in tins, and use of plastic containers has not been explored. The keeping quality of ghee is governed by such factors as the ripening of cream, method of manufacture, clarification temperature and the access which the packaging material affords to air and moisture. These aspects have been examined in the present study.

Materials and Methods

Ghee samples were prepared from unripened cow milk cream at 110°, 120° and 130°C in a double jacketed stainless steel kettle under controlled steam pressure. Ghee from ripened cow cream (0.3 per cent titratable acidity) was made at 120°C. Each type of ghee sample was made in 6 kg quantity and stored in 8 oz brown glass and screw cap low density polyethylene plastic bottles (Romoflex) in a dark place at ambient humidity (30-90 per cent) and temperature (16-32°C).

The samples were analysed for flavour and chemical changes at monthly intervals. Organoletpic evaluation was done by 5 trained panelists on an 8-point arbitrary scale where 8 denoted an excellent product and 2 or less an unacceptable one. Phospholipids were determined by the method of Rama Murthy and Narayanan[1], free fatty acids (FFA) and iodine by ISI: method[2] thiobarbituric acid (TBA) value by El-Negoumy and Hammond[3] and total carbonyl compounds by method of Rama Murthy and Jain[4].

Results and Discussion

Flavour and keeping quality of ghee: Effect of ripening of cream, clarifying temperature and packaging materials on flavour and keeping quality of ghee is shown in Table 1. Though the ghee samples were evaluated at monthly intervals, results are presented only for bi-monthly intervals. Ripening of cream to 0.3 per cent titratable acidity prior to clarification caused no flavour improvement in the resulting fresh ghee. The clarification temperature did show a definite effect on flavour of fresh ghee which was mild at 110°C and sharp at 120°C. Though ghee clarified at 130°C had a slightly cooked flavour, it was generally preferred by the panel members.

The flavour of ghee deteriorated with storage. The deterioration was fastest in ripened cream ghee followed by ghee clarified at 110°C. Clarification of ghee at a

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TABLE 1. EFFECT OF RIPENING OF CREAM, MANUFACTURING TEMPERATURE AND PACKAGING MATERIALS ON FLAVOUR* AND KEEPING QUALITY OF GHEE

Packaging material	Manuf. temp.(°C)	Ripening of cream	Storage period (months)						
			Initial	2	4	6	8	10	12
Glass bottle	110	No	5.0	5.0	4.5	4.0	3.5	2.0	—
,,	120	No	6.5	5.5	5.0	5.0	4.5	3.0	2.0
,,	130	No	7.0	6.0	5.5	5.0	5.0	4.0	2.0
,,	120	Yes	5.5	5.0	5.0	4.5	2.5	2.0	—
Plastic bottle	110	No	5.0	4.5	4.5	3.5	3.5	2.0	—
,,	120	No	6.5	5.5	5.0	5.0	4.0	3.0	2.0
,,	130	No	7.0	5.0	5.0	5.0	4.5	4.0	2.0
,,	120	Yes	5.5	5.0	5.0	4.0	3.0	2.0	—

*Ghee flavour intensity: 0–2 rancid; 2.5–4.0 flat or slightly oxidized; 4.5–6.0 mild; and 6.5–8.0 excellent. Average of two trials.

higher temperature resulted in a longer shelf-life; the shelf-life of ghee made at 120°C and 130°C was 10 and 11 months, respectively. The keeping quality of ghee packaged in plastic bottles was not different from that in brown glass bottles.

Lalitha and Dastur[5] also reported that ripening of cream for ghee manufacture adversely affected its keeping quality. Paul *et al*[6]. noted that quality of ghee kept in aluminium container was best and in a mud-pot, brass and bronze vessels, it was of worst quality.

Chemical changes: The initial free fatty acid content of fresh ghee ranged from 0.17 to 0.40 per cent; the lower limit was for ghee from unripened cream clarified at 110°C and the upper limit for ghee from unripened cream made at 130°C. The acidity level of ripened cream ghee made at 120°C was 0.32 per cent as against 0.18 per cent for unripened cream ghee at the same temperature (Table 2). The FFA content increased both by ripening and by heating to a higher temperature.

During storage for a year, the acidity of ghee increased reaching values from 0.30 to 0.59 per cent. The increase was largest in ripened cream ghee followed by sample made at lower temperature of 110°C. Acidity development was lower in ghee clarified at higher temperatures. Though the initial acid value of ghee made at 130°C (0.40 per cent) was higher than the final acid level of ghee made at 110 and 120°C after a year's storage, the flavour of the former was excellent whereas in the last

TABLE 2. EFFECT OF RIPENING OF CREAM, MANUFACTURING TEMPERATURE AND PACKAGING MATERIALS ON DEVELOPMENT OF FFA AND THIOBARBITURIC ACID VALUE OF GHEE DURING STORAGE*

Packaging material	Manuf. temp.(°C)	Ripening of cream	Storage period											
			Initial		2 months		4 months		6 months		8 months		10 months	
			FFA	TBA	FFA	TBA	FFA	TBA	FFA	TBA	FFA	TBA	FFA	TBA
Glass bottle	110	No	0.17	0.30	0.22	0.27	0.23	0.64	0.25	0.62	0.30	0.45	0.34	0.62
,,	120	No	0.18	0.52	0.20	0.62	0.25	0.85	0.25	1.00	0.30	0.55	0.30	0.33
,,	130	No	0.40	1.25	0.42	0.66	0.43	0.35	0.51	0.54	0.52	1.45	0.54	1.25
,,	120	Yes	0.32	0.20	0.36	0.63	0.39	0.77	0.45	0.38	0.57	0.58	0.59	0.74
Plastic bottle	110	No	0.17	0.30	0.20	0.93	0.23	0.37	0.25	0.44	0.35	0.39	0.36	1.65
,,	120	No	0.18	0.52	0.20	0.32	0.20	0.65	0.23	0.14	0.31	0.39	0.35	1.35
,,	130	No	0.40	1.25	0.42	2.30	0.43	0.68	0.48	0.50	0.50	0.47	0.52	0.95
,,	120	Yes	0.32	0.20	0.34	0.30	0.40	0.70	0.42	0.50	0.54	0.58	0.56	0.62

FFA = as % oleic acid

TBA = Thiobarbituric acid value = $\frac{(\text{O.D. sample} - \text{O.D. blank}) \times 10}{\text{Wt. sample (g)}}$

*Average of two trials.

TABLE 3. EFFECT OF RIPENING OF CREAM, MANUFACTURING TEMPERATURE AND PACKAGING MATERIALS ON PHOSPHOLIPID AND CARBONYL CONTENT OF GHEE DURING STORAGE*

Packaging materials	Manuf. temp.(°C)	Ripening of cream	Phospholipids (mg/100 g.)	Carbonyl contents* (μM/g) during storage (months)					
				Initial	2	4	6	8	10
Glass bottle	110	No	11.0	3.18	3.42	3.90	3.37	3.64	3.64
,,	120	No	34.3	3.33	3.42	4.20	4.66	3.64	3.69
,,	130	No	80.3	2.28	3.36	4.90	4.49	3.89	3.73
,,	120	Yes	45.3	3.56	3.69	4.40	5.07	3.64	4.08
Plastic bottle	110	No	11.0	3.18	3.33	4.00	4.22	3.11	3.56
,,	120	No	34.3	3.33	3.64	4.70	4.75	3.00	3.73
,,	130	No	80.3	2.28	2.76	4.50	4.75	3.64	3.91
,,	120	Yes	45.3	3.56	3.47	5.40	5.07	3.37	3.56

*Average of two trials.

two it was unacceptable. Free fatty acids liberated during ghee manufacture thus appear to get mixed up with the other flavouring components resulting in the development of a pleasant flavour. On the other hand, free fatty acids liberated or generated during storage alter the balance of flavouring components resulting in bad flavour. Thus a particular balance between flavouring components, acidic and otherwise, appear to determine the normal flavour of ghee. There was no significant difference in FFA content of ghee stored either in glass or plastic bottles.

Gaba and Jain[7] observed a 2-3 fold increase in acid value of ghee stored for 200 days at 37°C. However, Patel *et al*[8]. found no significant increase in the acid value of ghee stored for 6 months.

The TBA value of fresh ghee ranged from 0.2 to 1.25, the lowest for ripened cream ghee and the highest for unripened ghee made at 130°C (Table 2). The higher the clarifying temperature of unripened cream, the greater the rise in TBA value of the resulting ghee. There was a marked difference in the TBA values (0.52 and 0.20) of unripened and ripened cream ghee, though both were made at the same temperature of 120°C.

The TBA value showed erratic fluctuation during the storage period. Neither ripening, nor heating temperature, or type of packaging showed any consistent effect on the TBA value during storage. The TBA value, thus, appears to be unrelated to the consistent change in flavour and keeping quality of ghee. Similar observations were made by Hamm *et al*[9]. Mettler[10] and Fioriti *et al*[11]. As against this Gaba and Jain[3] reported a steady increase in TBA value of ghee during storage and a consistent relation with flavour deterioration.

The iodine value of fresh ghee ranged from 29.5 to 32.2 without any consistent relation to ripening of cream, heating temperature or packaging material. There was a slight general decrease in iodine number of ghee during storage, but the change was not related to the trend of deterioration of the product.

The phospholipid content of fresh ghee varied from 11.0 to 80.3 mg/100 g, the concentration being directly related to the clarification temperature (Table 3). Thus a higher phospholipid level has led, in unripened cream ghee, to improved storage. Ripening of cream led to a higher concentration of phospholipids in ghee, but not to an improvement in storage quality. These observations are in agreement with those of Rama Murthy *et al*[12]. and Rajput and Narayanan[13].

The total carbonyl content of the fresh ghee samples ranged from 2.28 to 3.56M μg/g. The ripening of cream did not affect the concentration of carbonyls. Its content was lowest (2.28M μ/g) in ghee made at the highest temperature (130°C). The concentration of carbonyl compounds increased slightly during storage, the increase being independent of ripening of cream, heating temperature and packaging material. Thus the total carbonyl content is not related to either flavour quality of fresh ghee or deterioration during storage.

Conclusion: The flavour and keeping quality of ghee appear to be mainly controlled by the clarification temperature used during preparation. The desirable initial ghee flavour intensity increased upto 130°C, and so was the case with subsequent keeping quality. Ripening of cream to 0.30 per cent titratable acidity prior to clarification did not affect the flavour, but did enhance its deterioration during later storage periods. Polyethylene plastic containers were not different from brown glass bottles for storage of ghee.

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Production and Storage of Buffalo Milk Cheddar Cheese in Paste and Powder Form

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A method for the preparation of cheese spread with about 58% moisture, 21% fat and 21% milk solids other than fat has been developed. It has also been possible to make this organoleptically acceptable product available in the form of a powder which could be reconstituted into cheese spread. This product could be stored at 37±1°C upto 6 months without the loss of desirable qualities.

Processed cheese prepared from a blend of differently aged cheese varieties is very popular. Cheese spread, commonly used as a paste for sandwitches, is another cheese based product used in Western countries. However, certain sections of the population in India are not used to strong cheese flavour. It was therefore, considered desirable to explore the possibilities of preparing a spread suitable for sandwitches or as a dip for biscuits containing cheese as base and mixed with butter/cream and other flavouring materials such as tomato pulp, mint, etc.

Taking into consideration the requirement of mild flavoured, smooth textured, cheese product for culinary use, the present investigation was undertaken to standardise the details for the preparation of cheese apread suitable for the Indian taste.

Olson *et al*[1]. have reported on the cheese spread made from two varieties of cheese namely Cheddar and Dariworld of normal and high pH. They observed that the normal Dariworld cheese was superior to normal Cheddar cheese. When cheese of high pH was used the spread gave undesirable firm body. Batch and Newman[2] in their patent used Cheddar cheese, milk fat, dried whey, dried skim milk powder and emulsifying agents. Panot[3] in his patent used unripened acid or rennet curd and emulsifying agents. Olson and Price[4] have reported about the preparation of cheese spread using cheese, butter, salt and emulsifying agents. The pH was maintained to 5.49±0.10.

Materials and Methods

The cheese spread was prepared by blending equal proportion of young (2 months old) and fully ripened (7-8 months) lots of buffalo milk Cheddar cheese (made according to the method of Burde's *et al*[5].). All the trials were conducted using 20 kg mix per batch. Minced cheese along with predetermined amounts of moisture, butterfat, salt and emulsifying agents (trisodium citrate and trisodium orthophosphate in the ratio of 5:1) at 2.0 percent were mixed thoroughly in a cheese processing kettle. Gelatin (0.3 per cent in dissolved form), flavouring agents like tomato juice, onion juice, etc. (optional at 5.0 per cent of the blend) were added at 60°C. The temperature was raised to 71°C with constant stirring and preservatives (nisin or sorbic acid or their equal proportion mixture) were added at the rate of 0.02 per

cent. The temperature was maintained at 71°C for 5 min with constant stirring. The hot cheese slurry was then homogenised in a single stage homogeniser at 100 kg/cm^2 pressure and collected directly in presterilized lacquered tin cans, covered with the lids and seamed immediately. The seamed cans were kept in hot water at 80°C for 5 min and then taken out and kept at room temperature for 24 hr. During this period, the cans were turned twice upside down after an interval of 8 hr.

A typical lot of 20 kg cheese spread was prepared with following ingredients:

Product	Qty.	Fat	Mois-ture	D.M.	Emulsifier
Cheese	10.43	2.92	3.96	3.54	—
Table butter	1.35	1.08	0.21	0.06	—
Water	7.82	—	7.82	—	—
Emulsifier	0.40	—	—	—	0.40
Total	20.00	4.00	12.00	3.60	0.40

Besides the ready to use form, cheese spread was also prepared in the free flowing powder form. For this purpose the homogenised cheese slurry without any added preservatives was adjusted to 35 per cent total solids and then spray dried at an inlet air temperature of 160°C and outlet air temperature of 100°C. This dried product was packed in lacquered tins under nitrogen packing.

Storage studies: Packed tins of paste and powdered cheese spread, were stored at 5°±0.5°C (RH 80±5 per cent), 30°±0.5°C (RH, 65±5 per cent) and 37°±0.5°C (RH, 60±5 per cent) and shelf life was assessed.

Chemical analysis of cheese blend, cheese-spread and cheese spread powder: Samples of cheese blend, cheese spread and cheese spread powder were analysed for fat, total solids, pH, salt, and total nitrogen by standard methods and non-protein nitrogen by the method proposed by El sokkary and Hassan[6]. The dried cheese spread was reconstituted by adding 55-60 per cent water (at 60°C). The temperature of the reconstituted product was increased to 70°-75°C with constant stirring. When desired consistency and shining appearance were attained, the product was transferred to the refrigerator for cooling.

After 4-6 hr, the reconstituted samples were examined and compared for body, texture and flavour with those of stored cheese spread (paste). Cheese spread was evaluated organoelptically by a trained panel, consisting of six judges. The samples were tested as acceptable or unacceptable on the basis of organoleptic evaluation.

Results and Discussion

The results on the quality characteristics of cheese spread blend are presented in Table 1. The mean fat and moisture content were 37.25 and 37.20 per cent respectively. The fat content was adjusted in such a way that the final product on dry matter basis tested had 50 per cent. The average pH of batches of cheese was 5.23, which is considered normal for Cheddar Cheese[7]. The values for total nitrogen and non-protein nitrogen showed negligible variations from the normal composition. Data pertaining to the analysis of the final product has been included in Table 1. The mean moisture content of the cheese spread was 57.35 per cent which was found to be quite suitable for spreading purposes. The mean fat content was 21.27 per cent which gives 50 per cent fat in dry matter as indicated above. The mean pH was 5.43. The mean total nitrogen and non-protein nitrogen contents were 2.73 and 0.48 per cent respectively.

The analytical values of cheese spread powder have been presented in Table 1.

The mean moisture content of the cheese spread powder was less than the prescribed limits of 3.5 per cent (for spray dried milk powder). The mean salt content of the powder was 2.0 per cent and the mean pH value was 5.32. The mean total and non-protein nitrogen was 5.38 and 1.00 per cent respectively.

The samples of cheese spread powder were found to be acceptable organoleptically even at the end of 6 months

TABLE 1. COMPOSITION OF CHEESE BLEND, CHEESE SPREAD AND DRIED CHEESE SPREAD

Particulars	Cheese blend Mean±S.E.	Cheese spread Mean±S.E.	Dried cheese spread Mean±S.E.
Fat%	37.25±0.44	21.27±0.49	54.04±0.80
T.S.%	62.70±0.73	42.34±0.34	96.63±0.28
Moisture%	37.20±0.72	57.35±0.29	3.37±0.28
Salt%	1.24±0.05	0.83±0.04	2.00±0.13
pH	5.23±0.03	5.43±0.03	5.32±0.03
Total N%	3.69±0.13	2.73±0.06	5.38±0.12
Non-protein N%	0.63±0.07	0.48±0.004	1.00±0.16

of storage at three temperatures employed in this study. The effects of adding preservatives such as nisin and sorbic acid on the shelf life was studied in the case of cheese spread (paste). On the basis of organoleptic evaluation, it was found that by the addition of nisin at 0.2 per cent (w/w), shelf life of the product could be enhanced from 15 to 30 days when stored at 37°C, from 20 to 45 days when stored at 30°C and from 40 to 90 days when stored at 5°C. Further improvement in the shelf life was observed when a mixture of nisin and sorbic acid (1:1) was employed at 0.2 per cent (w/w) in the cheese spread (paste). The shelf life of product in this case was found to be 55 days at 37°C, 90 days at 30°C and 120 days at 5°C. From these studies, it appears that under the tropical climatic conditions of India, dehydrated form of cheese spread powder will have better shelf life compared to the paste form.

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ERRATA

HEAT LOSSES FROM MILK SPRAY DRYERS by N. N. Varshney, A. N. Patil and T. O. Ojha, published in this Journal 1978, **15** (2), 81-85.

1. Page 83; line 28

$$q=A\triangle t\frac{1}{h}+\frac{x_1}{k_1}+\frac{x_2}{k_2}+\frac{x_3}{k_3}+\frac{x_4}{k_4}+\frac{1}{h_c-h_r}$$

should be read as

$$q=\frac{A\triangle t}{\frac{1}{h}+\frac{x_1}{k_1}+\frac{x_2}{k_2}+\frac{x_3}{k_3}+\frac{x_4}{k_4}+\frac{1}{h_c+h_r}}$$

2. page 83, Table 2 bottom foot note.

   The value for k is from ref (3)

   should be read as

   The value for k is from Ref (6)

3. page 83, equation No. 10 $h_c=1.12\ (\triangle t)\frac{1}{3}$

   should be read as

   $h_c=1.12\ (\triangle t^{\frac{1}{3}})$

RESEARCH NOTES

PROTEIN QUALITY OF NORMAL AND OPAQUE-2 MAIZE AT DIFFERENT STAGES OF RIPENING

In a nitrogen-balance study using albino rats, protein quality of immature normal and *Opaque*-2 maize were determined. Digestibility improved on ripening while biological value decreased. The decrease in biological value was greater in normal as compared with *Opaque*-2 Varieties. Biological value of immature normal maize was substantially higher as compared to the mature one. In addition, the biological value of normal maize at 25-day stage was as high as of *Opaque*-2 at maturity. Utilizable protein was higher in *Opaque*-2 as compared to normal maize at all the stages.

Maize, in India, is one of the important cereal crops grown throughout the country and most of the maize produced is consumed directly as human food. Green ears at milk or early dough stage are also consumed in roasted or boiled cotris, as snacks all over the country. In view of this it is desirable to gather information about the protein quality of mature and immature maize kernels. Earlier studies[1] showed higher levels of lysine and tryptophan in immature as compared to mature maize kernels. Present study involved the evaluation of nutritive quality of developing normal and *Opaque*-2 maize kernels by a nitrogen-balance study using albino rats. *Opaque*-2 maize was included in the present study because of its superior protein quality.

In the present investigation normal maize hybrid *Ganga*-5 and *Opaque*-2 composite *Shakti* were studied. Self-pollinated ears were harvested 25 and 35 days after pollination. The samples were dried in oven at 50°C. Protein was estimated by micro-Kjeldahl method[2] and tryptophan and lysine by colorimetric methods[3,4]. Nitrogen balance of each of the samples was determined in four Wistar male growing rats each weighing about 70 g, by the Mitchell and Carman method[5], as described by Lodha *et al*[6]. Each rat received daily 10 g dry matter, containing 150 mg N. Casein supplemented with 1 per cent methionine was used as control.

Results of analysis of *Ganga*-5 (Normal) and *Shakti* (*Opaque*-2) maize samples given in Table 1 show that during ripening protein, lysine and tryptophan contents decrease; the decrease was upto full maturity in *Ganga*-5 and upto 35 days post-pollination in *Shakti*.

True digestibility (TD), biological value (BV), net protein utilization (NPU) and utilizable protein (UP) values of immature kernels of normal and *Opaque*-2 are given in Table 2. True digestibility of immature normal as well as *Opaque*-2 kernels was considerably lower as compared to mature kernels. TD of normal and *Opaque*-2 kernels at 25 and 35 days post-pollination was respectively 16-18 per cent and 11-12 per cent lower as compared to the mature grains.

BV was substantially higher at 25-day stage compared to BV at maturity. In normal maize at 25-and 35-day stages, the BV were respectively 27.4 and 10.3 per cent higher compared to mature grains. BV for *opaque*-2 kernels was 13.8 and 2.6 per cent higher at 25-and 35-day

TABLE 1. PROTEIN, LYSINE AND TRYPTOPHAN CONTENTS OF MATURE AND IMMATURE KERNELS OF *Ganga*-5 HYBRID AND *Shakti Opaque*-2 MAIZE

	Ganga-5			*Shakti*		
	25 days	35 days	Mature*	25 days	35 days	Mature*
Protein (%)	13.44	12.50	11.81	12.69	11.69	11.69
Lysine (% in protein)	3.16	2.94	2.62	5.40	4.80	4.82
Tryptophan (% in protein)	0.57	0.49	—	0.80	0.76	—

*Values from the previous study[6]

TABLE 2. MEAN TRUE DIGESTIBILITY (TD), BIOLOGICAL VALUE (BV) AND UTILIZABLE PROTEIN (UP) OF MATURE AND IMMATURE KERNELS OF *Ganga*-5 HYBRID AND *Shakti Opaque*-2 MAIZE

		Ganga-5			*Shakti*		
	Control	25 days	35 days	Mature*	25 days	35 days	Mature*
TD	98.5±1.2	76.3±2.3	81.9±3.5	92.8±0.9	80.0±4.4	84.4±2.3	95.2±0.7
BV	90.9±1.0	76.3±4.2	70.8±3.0	59.9±0.8	86.5±3.7	78.0±4.5	76.0±1.3
NPU	89.5±2.9	58.2±2.4	58.0±4.5	55.6±1.2	69.2±1.7	65.7±3.4	72.4±1.6
UP	78.1±1.3	7.8±0.3	7.3±0.5	6.6±0.1	8.8±0.2	7.7±0.4	8.4±0.2

*Values from the previous study[6]

stages compared to at maturity. BV of unripe normal kernels at 25-day stage was comparable to that of mature kernels of *Opaque*-2.

NPU, which is a product of TD and BV, was found to be similar at 25-and 35-day stage for normal maize and it was slightly lower at maturity. NPU for *Opaque*-2 was higher compared to normal maize kernels at all the stages.

The utilizable protein $\left[\frac{(NPU \times Protein\ per\ cent)}{100}\right]$, which takes into account both protein quality and quantity, was 18.2 and 10.6 per cent higher respectively at 25-and 35-day stages as compared with mature grains in normal maize, whereas in case of *Opaque*-2, UP at 25-day stage was nearly comparable to that at maturity. Unlike normal maize, in *Opaque*-2, UP at maturity increased by 9 per cent in comparison to the value at 35-day stage. This is mainly due to the increase in the TD at maturity since no increase in protein content occurred between 35-day and mature stage (Table 2).

This study has, therefore, shown that during ripening the digestibility of proteins of both normal and *Opaque*-2 maize improves. The poor digestibility of immature kernels might be due to lower proportion of germ and higher proportion of pericarp unpublished data which contains crude fibre. The biological value decreased with ripening of the kernels; the decrease was more pronounced in normal as compared to *Opaque*-2. The study also indicates that the consumption of unripe normal kernels has significant nutritional advantage because of their improved nutritional quality. However, such additional advantage has not been observed in case of *Opaque*-2 maize.

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SIMPLE PROCEDURES FOR REDUCING THE COOKING TIME OF SPLIT RED GRAM (*CAJANUS CAJAN*)

Sodium carbonate and bicarbonate, trisodium phosphate and ammonium carbonate were tried as aids in reducing the cooking time of Red gram dhal (*Cajanus cajan*). Adding the chemicals singly or in combination to the cooking water was less effective than coating on the *dhal* and drying prior to cooking. Direct addition to cooking water increased the pH in the cooked products giving an alkaline taste. Soaking of the *dhal* in chemical solutions for 2 hr, draining and washing of the soaked *dhal* prior to cooking also brought about a desirable reduction in cooking time without altering the acceptability. Reduction in cooking time by about 50% by the above methods can be practised at home or in the *dhal* mills.

Whole legumes with intact husk are known to take a very long time to cook to a soft consistency. Dehusking and splitting into cotyledons (*dhal*) reduces the cooking time considerably.[1,2]. Even cooking of this *dhal* also takes long time (45 min to 1hr) in the case of red gram (*Cajanus cajan*). Reduction of cooking time of legumes by a simple process is, therefore, a consumer requirement in legume consuming countries. This is also of importance in the context of saving fuel.

Pressure cooking[2], addition of chemicals to the cooking water,[3,4], or soaking the legumes in solution of chemicals[5] have been practised for reducing the cooking time or producing quick cooking legumes. There is however, need for simple methods for reducing the cooking time which could preferably be adopted by *dhal* mills or at home. The suitability of 'chemical coating' procedure or a "chemical presoaking" method for reduction of cooking time of red gram *dhal* is reported here.

Two varieties of red gram *dhal*, one known for its short cooking time and another known to take a long time to cook were used. *Dhal* samples were first cooked in water to which chemicals ($NaHCO_3$, Na_2CO_3, $(NH_4)_2CO_3$ or Na_3PO_4) which had been shown earlier by Subba Rao *et al*[2]. to reduce the cooking time was added in different concentrations. In parallel studies the *dhal* was also given a coating of the chemicals by impregnation with solutions (5 ml/100 g) containing the same quantity of chemicals as added to the cooking water. After treating with the chemical solution the *dhal* was kept agitated for 5 min to prevent lumping caused by surface gums, allowed to equilibrate for 2 hr in a closed vessel and then dried in a current of air.

TABLE 1. EFFECT OF ADDITION OF CHEMICALS ON THE COOKING TIME OF RED GRAM

Chemical used	Level of addition (as %)	Cooking time of Red gram *dhal* Var 1*		Cooking time of Red gram *dhal* Var 2*	
		Chemical added to cooking water	Chemical coated	Chemical added to cooking water	Chemical coated
		min	min	min	min
None (Control)		60	—	45	—
$(NH_4)_2CO_3$	1	48	35	34	—
Na_3PO_4	1	45	38	33	28
Na_2CO_3	1	42	36	36	30
$NaHCO_3$	1	41	36	34	27
NaCl	1	65	60	46	43
Ammonia solution	(0.8% as NH_3)	—	44	—	—
$(NH_4)_2CO_3+NaHCO_3$	0.5+1.0	41	32	30	21
$(NH_4)_2CO_3+Na_2CO_3$	0.5+0.75	42	31	32	22
$(NH_4)_2CO_3+Na_2CO_3+NaCl$	0.5+0.75+1.0	46	32	35	24
$Na_3PO_4+(NH_4)_2CO_3$	0.5+0.5	44	35	—	—
$Na_2CO_3+(NH_4)_2CO_3$	0.5+0.5	40	34	—	—
$NaHCO_3+(NH_4)_2CO_3$	0.5+0.5	42	35	—	—

*Variety 1 is a commercially inferior variety as compared to Variety 2 because of its longer cooking time.

The time needed for cooking the *dhal* samples to a soft consistency was determined as described earlier[6].

Presoaking studies: The *dhal* was soaked for two hr in 1.5 parts of water to which the chemicals were added (0.75 to 1.5 g/100 g *dhal*), and the excess soak water was drained off. The soaked *dhal* was rinsed two times with water and cooked by adding to boiling water. Time needed for cooking, dispersed solids,

TABLE 2. COOKING CHARTACTERISTICS OF COATED RED GRAM DHALS

Chemical combinations	Level of addition as % of dhal	Cooking time (min)	Dispersed solids at 30 min, (%)	Water uptake (at 20 min) (g/g)	pH of the cooked dhal	Colour of the cook water	Taste of cooked dhal	Over all acceptability
Control (None)		60	47.8	0.82	6.30	Yellow	Normal	Acceptable
$Na_2CO_3+(NH_4)_2CO_3$	0.75+0.5							
Added to cook water		42	65.7	1.28	8.80	Deep brown	Alkaline	Not acceptable
Coated		31	88.3	1.33	8.50	Slight brown	alkaline	,,
$NaHCO_3+(NH_4)_2CO_3$								
Added to cook water	1+0.5	41	69.2	1.11	8.15	Brownish yellow	Sl. alkaline	,,
Coated		30	90.4	1.28	8.00	Sl. brown yellow	Sl. alkaline	,,
$Na_2CO_3+(NH_4)_2CO_3$								
Added to cook water	0.5 each	40	59.3	1.15	7.60	Yellowish brown	V. sl. alkaline	,,
Coated		34	81.0	1.20	7.30	V. faint brown	V. sl. alakaline	*
$NaHCO_3+(NH_4)_2CO_3$								
Added to cook water	0.5 each	42	63.2	0.95	7.50	Sl. Yellowish brown		*
Coated		35	79.8	0.98	7.20	Yellow	Normal	Acceptable

*Not acceptable as cooked *dhal* but acceptable in the form of *Rasam* or *Sambar*.

V. Sl: Very slight.

TABLE 3. EFFECT OF PRESOAKING OF RED GRAM DHAL IN CHEMICAL SOLUTIONS ON ITS COOKING TUME & OTHER CHARACTERISTICS

Chemical coimbinations	Level of addition (as % of dhal)	Variety 1				Variety 2			
		Cooking time (min)	Dispersed solids at 20 min (%)	Water up-take 10 min (g/g)	pH of the cooked dhal	Cooking time (min)	Dispersed solids at 30 min (%)	Water up-take 20 min (g/g)	pH of the cooked dhal
Control	Nil	40	49.8	1.0	6.6*	60	47.8	0.87	6.40*
Na_2CO_3	0.75	17	68.7	1.7	8.2**	34	64.0	1.70	8.15**
$NaHCO_3$	1.0	18	69.6	1.8	8.1**	27	55.6	1.90	7.95**
Trisodium phosphate	1.0	20	71.0	1.8	7.35*	38	58.2	1.68	7.30*
$(NH_4)_2CO_3$	1.5	18	67.3	1.9	7.1*	32	60.4	2.0	7.10*
$Na_2CO_3+(NH_4)_2CO_3$	0.75+0.5	15	86.5	1.9	8.3**	30	62.1	2.0	7.85**
$NaHCO_3+(NH_4)_2CO_3$	1+0.5	15	86.1	1.9	8.1**	25	63.0	2.10	7.90**
Trisodium phosphate $+(NH_4)_2CO_3$	1+0.5	19	70.0	1.7	7.7**	27	51.7	1.85	7.45**

*Acceptable in taste and flavour

**Alkaline in taste and unacceptable as '*dhal*' but acceptable with tamarind adjunct in 'Rasam' & 'Sambar'

hydration during cooking, pH and acceptability of cooked *dhal* samples were determined as described earlier[6].

As a combination of sodium bicarbonate and ammonium carbonate or sodium carbonate and ammonium carbonate was found to reduce the cooking time by about 50 per cent, 10 kg batches of the hard cooking variety of *dhal* were coated with these chemicals at two levels. The cooking characteristics of the coated *dhal* as compared with the control were determined[6].

The chemicals were more effective in reducing the cooking time when coated on the *dhal* than when added to the cooking water (Table 1). Trisodium phosphate or ammonium carbonate along with sodium carbonate and bicarbonate applied to the *dhal* by the coating technique brought about nearly 50 per cent reduction about in the cooking time. Trisodium phosphate brought tenderisation of texture but was found to leave a lingering taste on the tongue. Hence only combinations of ammonium carbonate with sodium carbonate or bicarbonate were studied.

Data on the cooking characteristics of *dhal* treated in bulk with the latter combinations at two levels are presented in Table 2. While coating treatment with the chemicals reduced the cooking time by about 50 per cent, there was slight discolouration of the cooked *dhal* and an alkaline taste (pH above 7.5) at the higher level of chemicals added. However, at the lower level of chemicals (0.5 per cent) the cooked *dhal* was acceptable (pH about 7.2). When used for preparation of 'Rasam' or 'Sambar' the cooked materials were found to be quite acceptable even at the higher level of addition of chemical. The tamarind used for acidification in the above prepations neutralised the alkalinity of the chemicals. Dispersed solids and the water uptake which are parameters of cooking quality were greater in the case of coated *dhals* than the untreated *dhal* or *dhal* when cooked in water containing the same amount of chemicals.

Presoaking the *dhal* for 2 hr in the chemical solutions brought down the cooking time by nearly 50 per cent in both the varieties of *dhal* (Table 3). Although all the chemicals used were effective in reducing cooking time and increasing the proportion of dispersed solids and hydration during cooking as compared with the control, ammonium carbonate and trisodium phosphate appeared to be more useful than others. The other chemicals either singly or in combinations gave alkaline or other undesirable tastes in the cooked product.

The above studies bring out the possibility of reducing the cooking time of red gram *dhal* by two simple methods using cheap and permissible chemicals. The coating technique can be carried out in bulk in *dhal* mills as an end step in the *dhal* milling process while the presoaking procedure can be practised at home by the housewife prior to cooking. The coating procedure has also been found to be suitable for cooking of bean *dhal* (*Phaseolus vulgaris* or *Rajma*) which is known to take a long time to cook.

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EFFECT OF TURMERIC (*CURCUMA LONGA*) ON THE GROWTH OF SOME INTESTINAL BACTERIA *IN VITRO*

Effect of turmeric on the growth rate and acid production by some intestinal bacteria was examined. It was found that turmeric suppresses the growth and acid production by *Lactobacilli* while it slightly stimulates the growth and acid production by *Streptococci* and *Escherichia coli*. Minimum effective concentration of turmeric was found to be 0.5%.

In traditional medicine, turmeric has an important place in the treatment of many physical ailments and surface wounds. Ramprasad and Sirsi[1], Chopra *et al*[2]. and Subba Rao *et al*[3]. have shown different properties of different fractions of turmeric. Nadakarni[4] ascribed a number of medicinal properties for turmeric. So far no rational explanation has been offered to these properties except for a few of them. The present communication is an attempt to understand the effect of turmeric on intestinal microflora which have an important role in the health of the host animals.

Nutritent broth with 0.5 per cent glucose and nutrient agar medium was used for growth and plate count of *Escherichia coli* and enrichment medium for lactic cultures was used for lactic acid bacteria. In all the media, 0.5 per cent bacto bile salts were included to provide the conditions more akin to that of intestine.

The effect of turmeric was tested on *Lactobacilli*, *Streptococci* and *Escherichia coli*. 200 ml of the respective media with 0.5 per cent bile salts was divided into two equal portions of 100 ml, turmeric (0.1 to 1g) was added to one portion and stirred well. The pH of both the portions was adjusted to 7.2 separately and autoclaved at 15 lb pressure for 20 min; 0.5 ml of inoculum from 24 hr old culture having good growth of the bacteria was added to each portion and incubated at 37°C. The 100 ml portion without turmeric served as control. At different intervals 3 ml medium was drawn from each portion and titrated against 0.05 N NaOH, with a few drops of 0.04 per cent bromothymol blue as indicator, to test for acid production. Simultaneously 1 ml of the culture medium from each lot was drawn for taking plate count.

Both *L. acidophilus* and *L. plantarum* were found to be sensitive to turmeric (Table 1).)But, a higher titre value was seen in turmeric added medium, at the initial stages which was probably due to interference of the yellow colour of the turmeric in the titration. Even though titre value was higher, plate count was found to be lesser in turmeric medium which confirmed the colour interference in the titration.

Growth of *S. faecalis* was enhanced by turmeric, but that of *S. lactis* was retarded upto 24 hr after which it gained normal growth as indicated by acid production and plate count (Table 2). Our results with *E. coli* were in agreement with the findings of Ramprasad and Sirsi[1] who tried to see the effect of curcumin and essential oil of turmeric on some bacteria. Turmeric did not inhibit *E. coli* at 1 per cent concentration, but on the other hand, was slightly stimulatory (Table 2). Probably, this may be the reason why Subba Rao *et al*[3]. did not find any change in the total count of caecal microorganisms of rats fed curcumin, even though there might be growth retardation of some other bacteria.

L. acidophilus and *L. plantarum* showed their sensitivity at 0.25 per cent of turmeric concentration in the medium. But the retardation of growth was slight at

TABLE 1. EFFECT OF TURMERIC ON THE GROWTH RATE AND ACID PRODUCTION OF *L. Acidophilus* AND *L. Plantarum*

Organism	Incubation period (hr)	Acid produced[a]		Plate count (cells/ml)	
		Control	Turmeric treated	Control $\times 10^4$	Turmeric treated $\times 10^4$
L. acidophilus	8	0.55	0.65	140	23
	24	1.05	0.65	400	16
	48	1.45	0.65	4500	6
L. plantarum	8	0.40	0.60	33	11
	24	0.55	0.65	860	10
	48	1.40	0.65	7300	4

[a]ml of 0.05 N alkali/3 ml medium

TABLE 2. EFFECT OF TURMERIC ON THE GROWTH RATE AND ACID PRODUCTION OF *S. Faecalis S. Lactis* AND *E. Coli*

Organism	Incubation period (hr)	Acid produced[a]		Plate count (cells/ml)	
		Control	Turmeric treated	Control $\times 10^8$	Turmeric treated $\times 10^8$
S. faecalis	8	0.35	0.45	16	21
	24	1.25	1.45	760	2900
	48	2.15	2.65	930	4300
S. lactis	8	0.70	0.65	1.9	1.8
	24	2.00	1.80	80	60
	48	2.60	2.60	960	880
E. coli	8	0.80	1.15	5.8	7.8
	24	1.05	1.55	7.5	15
	48	1.25	1.65	10	35

[a]ml of 0.05 N alkali/3 ml medium

TABLE 3. EFFECT OF DIFFERENT LEVELS OF TURMERIC ON *L. ACIDOPHILUS* AND *L. PLANTARUM* (AFTER 48 HR. OF INCUBATION)

Turmeric concn. (%)	*L. acidophilus*		*L. plantarum*	
	Titre value[a]	Plate count (cells/ml $\times 10^5$)	Titre value[a]	Plate count (cells/ml $\times 10^4$)
Nil	1.30	5300	1.15	6800
0.10	1.30	5200	1.15	5800
0.25	1.15	4500	1.10	2400
0.50	0.65	34	0.65	100
0.75	0.55	8	0.55	4
1.0	0.55	8	0.55	1

[a]ml of 0.05 N alkali/3 ml medium

that concentration, while the significant and almost complete retardation was found at concentration of 0.5 per cent and above. (Table 3). Hence, it can be concluded that turmeric can alter the intestinal microflora by inhibiting or enhancing the growth of different intestinal bacteria and 0.5 per cent of the turmeric is the minimum concentration necessary to bring about the effect on bacterial growth.

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DETERMINATION OF THEOBROMINE IN COCOA PRODUCTS

Four existing methods of estimating theobromine in cocoa products were evaluated using samples of cocoa powder and drinking chocolate. Gerritsma and Koers method was found simple, accurate and suitable for routine analysis of cocoa samples.

Theobromine is the most important alkaloid of the cocoa-bean, in which it occurs to the extent of 1.8 per cent. Besides the stimulant action, it has a mild physiological action on the nervous system and kidneys. It has a considerable practical use as a diuretic. The determination of theobromine in cocoa products, is therefore of much importance.

In practice, however, the determination of theobromine in cocoa products has always been difficult. A number of gravimetric and volumetric methods have been suggested from time to time. Four of these methods[1-4] were selected for comparative study. Four samples of cocoa powder and three of drinking chocolate were analysed using these methods, and the results are given in the Table 1. (The estimations were carried out in triplicate).

TABLE 1. THEOBROMINE CONTENT OF COCOA POWDERS
(Summary of the Statistical Analysis)

	Mean Thebromine content per cent (Dry fat free basis)	Coefficient of variation
1) Holmes method	1.76*a*	67.19
2) Gerritsma and Koers method	1.81*ab*	66.76
3) Moir and Hinks method	1.83*ab*	64.44
4) Spectrophotometric method	2.07*b*	63.49
Standard error of Mean (18 *df*)	±0.09	

Means of the columns followed by different letters differ significantly at 5% level.

The results indicate that the values for theobromine obtained by methods 1, 2 and 3 are in close agreement. Statistical analysis shows that the difference between the methods 1, 2 and 3 is not significant at 5 per cent level, whereas it is significant between the methods 1 and 4. The coefficient of variation ranges from 63.5 to 67.2, among the four methods.

Holmes and Moir and Hinks methods are somewhat tedious and time consuming. Holmes method also involves use of a specially designed liquid-liquid extractor. The U.V. Spectro-photometric method gives somewhat higher values, attributable to the absorption by unknown components of cocoa in the U.V. region.

We find the Gerritsma and Koers method simple, giving reproducible results. A number of samples can be analysed in a short period and the method is very well suited for routine analysis of cocoa and cocoa products.

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DETECTION OF AMBADI (*HIBISCUS CANNABINUS*) SEED OIL IN VEGETABLE OILS

TLC of the unsaponifiable matter of ambadi seed oil (*Hibiscus cannabinus*) using benzene-absolute alcohol-acetic acid (95:2.5:2.5 v/v) as solvent, followed by spraying with ortho-phosphoric acid-water (2:3 v/v) and revealing by heat, yields a characteristic blue spot at Rf 0.15. This enables its detection at 5 per cent level in other oils, including cottonseed oil which itself contains cyclopropene fatty acids.

Ambadi (*Hibiscus cannabinus*) has about 22 per cent oil, and potential for oil production is placed at about 13,000 tonnes annually[1]. *Ambadi* oil contains 5-15 per cent of cyclopropene fatty acids[2,3] about five to ten times as much as does cottonseed oil. Hence use of the Halphen test, which is based on these constituents[4], would not permit its detection if cottonseed oil is present. A method is described based on TLC resolution of the unsaponifiable matter (UM) that can detect *ambadi* oil in cottonseed or other oils at 5 per cent level.

Usual TLC plates of silicagel G were employed[5]. The UM from pure and mixed oils (1.5 ml) was extracted with ether (20 ml. 10 ml) after saponification (10 ml of 10 per cent alcoholic potash) and dilution (20 ml water), followed by thorough washing of the ethereal extract and drying over anhydrous sodium sulphate. The UM was dissolved in 1.5 ml chloroform (containing 2% acetic acid) and 20 ml spotted; the plate was developed with benzene—absolute alcohol-acetic acid (95:2.5:2.5 v/v), dried in an oven for a few minutes, sprayed with ortho phosphoric acid:water (2:3 v/v) and heated at 100°C for 10 min.

Fig. 1 shows that all oils exhibited 4-5 spots that differed in colour between Rf 0.5 and 1.0, representing various constituents of the UM. The UM of *ambadi* seed oil was characterised by a specific prominent blue

Detection of *ambadi* seed oil in the other vegetable oils in presence of cottonseed oil.

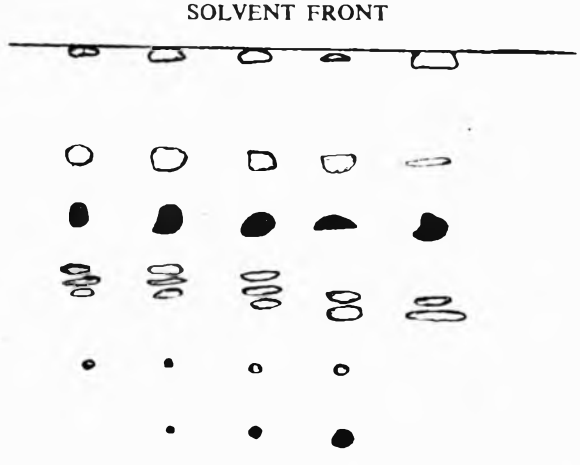

Fig. 1. Chromatogram of U.S.M. of different vegetable oils resolved on T.L.C. plate.

concentration from other faint spots of various colours spot at Rf 0.15, easily recognsiable even at 5 per cent (including bluishhue) at about this Rf shown by other oils.

Expressed and solvent-extracted *anbadi* oils, in both unrefined and refined forms, all yielded this characteristic UM spot. No such spot was given by the UMs of cottonseed, silk cottonseed (*Bombax malabariam*) tobaccoseed, vanaspati and many other oils examined. A level of 5 per cent of *ambadi* seed oil could be detected in any of the seed oils and vanaspati samples examined.

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TOXICITY OF METHYL IODIDE TO SOME LIFE STAGES OF *EPHESTIA CAUTELLA* WLK. (PYRALIDAE), *TRIBOLIUM CASTANEUM* HERBST. (TENEBRIONIDAE), AND *TROGODERMA GRANARIUM* EVERTS. (DERMESTIDAE)

Methyl iodide (MeI) used as a fumigant against life stages of *Ephestia cautella* Wlk., *Tribolium castaneum*, Herbst., and *Trogoderma granarium* Everts, showed the following order of tolerance of the insect growth stages. *T. castaneum larva* > *T. granarium* larva > *E. cautella* larva > *T. castaneum* pupa > *T. castaneum* egg at both the LD_{50} and LD_{95} levels. Unlike the general trend shown by fumigants the pupal and egg stages were the least tolerant to MeI.

Earlier work on toxicity of methyl iodide (MeI)[1] to stored product insects was confined to five species of adults. No work on this fumigant has been reported on other life stages of these insects. The present investigation was undertaken to study the toxicity of MeI to the life stages of three species of insects which breed outside the grain, i.e., in intergranular space.

The following test insects were used:

Species	*Life stage*	*Age*	*Rearing medium* (25±1°C)
Ephestia cautella wlk.	Larva	2nd instar	Broken groundnuts
Tribolium castaneum Herbst	Eggs	1-2 day	Whole wheat flour
	Larvae	2nd instar	,,
	Pupae	2-3 day	,,
Trogoderma granarium Everts.	Larvae	2nd instar	Broken wheat (35°C)

The *E. cautella* and *T. granarium* larvae were exposed in open end glass tubes with cloth diaphragm closures and others were exposed in open petri dishes.

For collecting the eggs of *T. castaneum*, adult beetles (older than 3 weeks) were allowed to lay eggs for 24 hr in whole wheat flour (sieved through 40 mesh standard screen) fortified with 5 per cent brewer's yeast. The adults were then removed by sieving through 30 mesh standard screen, retaining the eggs on the 40 mesh screen.

The fumigation procedure and conditions were as described earlier[1] i.e. in 2.5 l. glass desiccators, along with controls, at a temperature of 25±1°C and 65 per cent R.H. with an exposure period of 24 hr. Egg mortality was computed taking the number that emerged from the controls as 100 per cent (alive). After preliminary trials, 3-5 dosages were examined with 5-7 replicates per dosage, with a minimum of 30 insects per replicate. Mortality was assessed and the data were analysed by the method of Litchfield and Wilcoxon[2].

The LD_{50} and LD_{95} values (mg/l), the slope function, and the 95 per cent confidence limits are shown in Table 1. The order of tolerance to MeI was *T. castaneum* larva > *T. granarium* larva > *E. cautella* larva > *T. castaneum* pupa > *T. castaneum* egg at both LD_{50} and LD_{95} levels. There was no mortality in controls.

An examination of the slope function showed that the log dose probit lines were almost parallel for all the insect stages tested except *T. castaneum* eggs where higher increments of dosage were required per unit mortality.

Compared to methyl bromide (MB)[3,4] MeI appears to be less toxic to *T. castaneum* larvae, but more, toxic to the pupae and eggs. *T. granarium* larvae and *E. cautella* larvae also appear to be less tolerant to MeI than to MB. Generally, the pupae and possibly the eggs of *S. oryzae*, are the most tolerant to several fumigants[5]. This is also true more or less for other stored-product insects. But, the data obtained with MeI in the present study show that the pupa and the egg are the least tolerant. This high toxicity of MeI to pupae and eggs

TABLE 1. TOXICITY OF METHYL IODIDE TO SOME LIFE STAGES OF *E. Cautella*, *T. Castaneum* AND *T. Granarium* EXPOSED FOP 24 HOURS

Insect Spp.	Life stage	LD_{50}(mg/l)			LD_{95}(mg/l)			Slope		
		Value	95% limits		Value	95% limits		Value	95% limits	
			Upper	Lower		Upper	Lower		Upper	Lower
Ephestia cautella	Larva	1.5	1.6	1.4	2.1	2.5	1.8	1.2	1.3	1.1
Tribolium castaneum	Larva	2.7	3.2	2.2	4.2	5.9	3.0	1.3	1.5	1.1
	Pupa	0.29	0.34	0.24	0.62	0.87	0.44	1.58	1.88	1.18
	Egg	0.05	0.06	0.04	0.12	0.16	0.09	2.10	2.40	1.80
Trogoderma granarium	Larva	2.0	2.4	1.7	3.8	5.7	2.5	1.5	1.9	1.2

is of great practical value in fumigation. In a treatment, if the adults and larvae succumb, it can be safely concluded that the eggs and pupae have also been killed.

In both the egg and the pupa, fat is usually the chief reserve substance which provides energy for growth. Selective consumption of unsaturated fat is found in some Lepidoptera[6]. It is possible that the iodine moiety of MeI which is very reactive may combine with the fat, thus poisoning the chief energy substrate. The adults and larvae with their higher rates of respiration will probably be able to detoxify the MeI imbibed, more effectively. Carlson[7] has suggested that the toxicity of MB in the case of *T. castaneum* is inversely proportional to respiratory rate. Winteringham[8] has reported only partial destruction of reactive thiol (reduced glutathione) group *in vivo* in *Calliphora* larvae with MeI, as compared to MB, where total destruction occurred.

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EVALUATION OF QUALITY AND GRADING OF DRIED MILKS

A total of 216 dried milk samples comprising 124 spray dried, 54 roller dried and 38 infant milk powders were collected from different sources. The dried milks were reconstituted and stored. Their quality was assessed on the basis of organoleptic and microscopic tests. Samples of Spray dried and infant milk powders were graded into four categories namely, excellent, good, fair and poor. Roller dried samples could not be graded because of lower solubility and more whey separation.

Dried milks after reconstitution are presently distributed in the urban areas[1]. Quality of the milk powder determines storability and final stability of the reconstituted milk. Present methods of assessment are based on establishing the standards for the dried milk powders meant for this purpose. Although the plate count method has been recommended for assessing the quality of milk powders[2], it has been found to be of less value since the stored samples of dried milks yield lower counts[3,5]. According to the earlier workers[4,6], it would be advantageous to adopt a suitable grading system for spray dried milk in order to judge its quality based on parameters other than plate counts. Likewise, Leighton[7] has suggested a method for grading the spray dried milk powder and noticed that the product can be distinguished from good and bad. The present study was, therefore, aimed at evaluating the quality and grading of dried milk samples so that the present information together with the plate count method can be used in formulating the standards for quality control purposes.

The experiments were conducted during 1975-76 on freshly prepared reconstituted milk samples from spray and roller dried milk powders and infant milks. Leighton's[7] method was modified to classify the dried milk products, into four different grades namely 'excellent', 'good', 'fair' and 'poor'. Ten grams of dried milk sample was weighed into a 2 oz sterile sample bottle. To this, 100 ml of sterile and warm (45°C) distilled water was gently added and the contents were shaken thoroughly for mixing[2]. The reconstituted milk samples were stored for 48 to 72 hr at 22°C for the development of spontaneous acidity and coagulum. During the first 24 to 48 hr, whenever there was no coagulum formation the contents in the bottle were gently shaken (swirled) to ensure uniform development of acidity and coagulum.

The stored samples of reconstituted spray dried, roller dried and infant milk powders were examined by Gram's staining technique and the type of curd formation according to the standard procedures[8,9]. The above method was not applicable to roller dried milk powders as the reconstituted milks presented several difficulties like poor solubility, whey separation and weak coagulum.

Among the 124 samples of spray dried milk examined, 10 were graded as 'excellent'. The curd exhibited an acidic or sour smell with a good firm body without any excess whey formation or gas holes. In a similar manner, 15 out of 38 samples of infant milk powders showed the above characteristics and therefore were placed in 'excellent' category. Gram positive, small thick rods occurring in singles or chains followed by thin rods and cocci in pairs were observed in the curd samples.

A total of 12 spray dried and 19 infant milk powder samples were classed 'good'. The organoleptic and bacteriological qualities were similar to those of 'excellent' grade, except that there was little whey formation and very few Gram negative rods. A majority of spray dried milk samples (80) were placed in 'fair' class and only 4 samples of infant milk powder belonged to this category. The curd samples exhibited a fruity or fishy and unpleasant smell with a semi hard curd and whey formation. The predominant types of bacteria were similar to those found for 'excellent' grade and the other types of bacteria were more in numbers.

The 'poor' quality samples exhibited an offensive or bad flavour with a blown up curd floating on the top and accumulation of more gas bubbles. Twenty two (17.7 per cent) samples of spray dried milk belonged to

TABLE 1. QUALITY EVALUATION AND GRADING OF DRIED MILKS AFTER RECONSTITUTION AND STORAGE

Grading	Organoleptic quality of curd	Microscopic examination of curd				Distribution of samples			
						Spray dried		Infant milks	
		1	2	3	4	No. of samples		No. of samples	
Excellent	Acidic/sour, pleasant, good firm curd (when cooled to 7°-10°C). No whey, no gas holes.	Gram+ve Small, thick, rods in single or chains.	Gram+ve, long, thin rods (few)	Gram+ve, cocci in pairs (few)	Nil	10	8.1	15	39.5
Good	Acidic/sour, pleasant, good firm curd (when cooled to 7°-10°C). No or little whey, no gas holes.	,,	,,	,,	Gram-ve, thin, short, rods (very few).	12	9.7	19	50.0
Fair	Fruity/fishy, pleasant/unpleasant, semi hard curd with whey formation (occasionally gas bubbles).	,,	Gram+ve long, thin, rods (more)	,, (more).	,, (few).	80	64.5	4	10.5
Poor	Offensive/bad, blown up curd floating on top accumulation of more gas bubbles).	Gram+ve cocci in pairs or small chains.	Gram+ve cocci in long chains.	Gram+ve short, thick, rods in single or chains.	,, (more).	22	17.7	NIL	NIL

1,2,3,4—Order of predominance of the organism.
Observations recorded are from the duplicate samples.

this group while no 'poor' sample of infant milk was detected. The various types of bacteria found in spray-dried curd samples are given in Table 1. In a similar study on spray dried milk in England, Nicohls[6] reported that 90 per cent of the total samples (400) examined after reconstitution and storage for $2\frac{1}{2}$ to $3\frac{1}{2}$ days at 15.6°C were sweet.

Since Indian Standards specification specifies only total bacterial counts and limits of coliform counts, it is possible to introduce organoleptic and microscopic evaluation tests for further grading of acceptable quality of milk powders into different grades as described in this paper.

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PRESERVATION ASPECTS OF KHOA IN RELATION TO USE OF RADIO-STERILIZED FLEXIBLE PACKAGES

Asceptic packaging of *khoa* in radiation sterlized laminated pouches extended the shelflife to 7-22 days as against 1-3 days for unpacked samples when stored at 37°C. Similar results were also observed with the use of unsterlized pouches, indicating insignificant microbial contamination of the product from the packaging material. The shelf life of the product remained unaffected by vacuum packaging.

Khoa or *mawa* the most important indigenous milk product and sweet base, though supposed to be free from great majority of microflora present in the milk which are destroyed during its preparation, gets spoiled due to subsequent contamination from improper handling and its unhygienic marketing conditions. The product deteriorates due to degradation of its major constitutents like lactose, fat and proteins[1] and thus has poor shelf-life Despite the use of butter-paper packaging and addition of cane sugar[6] and application of ultra violet irradiation[3] only marginal improvement in its shelf-life has been possible. Since packaging material is presumed to be one of the sources of microbial contamination the study on the use of radio-sterilized packages on the shelf stability of the product was undertaken.

Four batches of *khoa* samples prepared[5] in laboratory from cow's milk were transferred asceptically to sterilized containers. About 250 gms. of the product was packed hot (70 to 80°C) in each of the two types of irradiation-sterilized laminated-pouches (5.5″ × 8″) as below alongwith the control under asceptic conditions:

i) Polyethylene (0.051 mm) / 300 PT—cellophane

ii) Polyethylene (0.038 mm) / aluminium foil (0.009 mm) / polyethylene (0.025 mm) / paper (80 GSM).

The pouches were radio sterilized at 2.5 Mrad using Co_{60} source of Bhabha Automic Research Centre (B.A.R.C.)

The pouches were air sealed using electric sealer and vacuum sealed on "Auto-Vac" machine which is semi-automatic and capable of vacuum sealing by impulse heat. The sealed pouches were stored at 37°C in incubator considering the high local temperature around Bombay.

The packed samples were evaluated weekly organoleptically by technical panel members for preference/acceptability test using 9—point hedonic scale[11] and off-odour, irradiation flavour and off-flavour on 9 point intensity scale.

The product scoring 5 or more on hedonic and 5 or less on intensity scale was considered acceptable.

Moisture content and acidity (oleic) were determined by ISI methods, (ISI:1964). Peroxide value and colour-index were determined colorimetrically. Thiobarbituric acid value (TBA) was also determined colorimetrically as per Mehlenbachen[9]. Standard plate count and yeast and mold count were determined as per APHA[2].

Initial values for acceptability score, peroxide value, free fatty acids (oleic) were 7.5, 0.075 m mol O_2/Kg. and 0.032 per cent respectively. The SPC as well as total counts for yeast and mold for fresh *khoa* were found to be less than 10 per gram.

Chemical as well as organoleptic indices for *khoa* packaged in 2 or 4 ply laminated pouches were not

TABLE 1. EFFECT OF AIR AND VACCUM PACKAGING ON QUALITY OF *Khoa* (AVERAGE VALUES)

Packaging Material	Radiation treatment of pouches	Storage period, days	Vacuum packaging					Air packaging				
			Acceptability score	P. V. m mol O_2/kg	FFA %	SPC/g	Yeast & mold/g	Acceptability score	P. V. m mol O_2/kg	FFA %	SPC/g	Yeast & mold/g
2 ply[a]	Nil	12	6.0	0.208	0.184	3.0×10^4	2.0×10^2	5.0	0.342	0.160	4.0×10^4	<10
	Sterilized	14	5.0	0.339	0.138	8.0×10^2	<10	5.0	0.339	0.134	1.8×10^8	<10
4 ply[b]	Nil	22	5.8	0.169	0.230	$>5.0\times10^6$	10^2	5.2	0.340	0.380	1.6×10^2	10
	Sterilized	22	5.8	0.052	0.240	$>5.0\times10^6$	10	5.2	0.250	0.340	$>3.0\times10^6$	20

P. V.: Peroxide value, F.F.A.: Free fatty acids (as oleic acid) S.P.C.: Standard plate count.
a: Polyethylene/cellophane. *b*: Paper/polyethylene/foil/polyethylene.

affected by vacuum packaging. Hence, discontinued and further studies were made on with samples packed under atmospheric conditions, (Table 1). The environment of packaging does not influence shelf stability of milk-based product as reported by Date and Bhatia[4] as nitrogen packaging of *burfee* did not prolong its keeping quality. Naidu and Ranganathan[10] also observed the lack of co-relation between organoleptic attributes and microbial load of market samples of *khoa* as also seen in this study that samples having high bacterial counts (more than 10^6/g.) were also considered acceptable even after 7 days storage.

Chemical indices of *khoa* packed in laminated pouches have been presented in Table 2, loss of moisture from *khoa* during storage was observed in both types of packing to varying degrees but this was more as anticipated in 2 ply than 4 ply laminated pocuhes in all the four batches of samples. The factors like peroxide value, free fatty acids and TBA increased with storage, irrespective of the type of pouch and sterilization treatment, indicating onset of oxidative changes in the product. The changes were, however, not enough to cause objectionable rancid flavour upto a period of three weeks, which was substantiated by lower scores for off odours

TABLE 2. CHEMICAL INDICES OF *KHOA* PACKAGED IN LAMINATED POUCHES AND STORED AT 37°C

Batch No.	Package type	Radiation treatment of package	Shelf life (days)	Initial Values: Moisture, (%)	Colour index	Peroxide value (m mol O_2/kg.)	FFA (as oleic acid), %	TBA (mg malonaldehyde /kg)	Terminal Values: Moisture, (%)	Colour index	Peroxide value (m mol O_2/kg.)	FFA (as oleic acid), %	TBA (mg malonaldehyde/ /kg.)
1.	2 ply[a]	Nil	7	31.7	3	0.030	0.260	0.000	27.5	5	0.990	0.480	0.175
	Sterilized	Sterilized	7	32.0	3	0.30	0.260	0.000	27.5	6	1.280	0.360	0.180
2.		Nil	14	39.6	3	0.500	0.280	0.000	35.6	4	0.600	0.360	0.350
		Sterilized	14	39.6	3	0.500	0.280	0.000	35.6	4	1.020	Nil	0.350
3.		Nil	21	35.4	4	0.090	0.240	0.000	31.5	6	0.300	0.280	0.175
		Sterilized	21	35.0	4	0.090	0.240	0.000	30.4	6	0.300	0.360	0.175
4.		Nil	24	33.4	3	0.120	0.160	0.170	30.6	7	0.252	0.362	1.070
		Sterilized	24	33.8	3	0.128	0.160	0.170	30.3	5	0.230	0.400	0.659
1.	4 ply[b]	Nil	7	30.7	3	0.030	0.260	0.000	27.5	5	0.620	0.600	0.00
		Sterilized	7	30.9	3	0.030	0.260	0.000	27.5	—	0.820	0.560	0.180
2.		Nil	7	39.6	3	0.500	0.160	0.350	37.2	4	1.220	0.280	—
		Sterilized	7	39.0	3	0.500	0.280	0.350	36.0	6	0.520	0.320	0.530
3		Nil	14	35.0	4	0.090	0.240	0.175	33.9	8	0.180	0.400	—
		Sterilized	14	35.8	4	0.090	0.240	0.170	35.0	7	0.150	0.440	0.350
4.		Nil	14	33.2	3	0.120	0.160	0.170	32.1	5	0.202	0.480	0.700
		Sterilized	14	33.4	3	0.120	0.160	0.170	32.1	5	0.235	0.440	0.700

a) Polyethylene/cellophane;
b) Paper/polyethylene/foil/polyethylene.

TABLE 3. ORGANOLEPTIC AND MICROBIAL PATTERNS OF *Khoa* PACKAGED IN LAMINATED POUCHES AND STORED AT 37°C.

				Initial Values					Terminal Values				
				Microbial analysis		Sensory score			Microbial analysis		Sensory score		
Batch No.	Package type	Radiation treatment of packages	Shelf life (days)	Total plate count/g	Yeast and mold/g	Accept-ability	Off odour	Off flavour	Total plate count/g	Yeast and mold/g	Accept-ability	Off odour	Off flavour
1.	2 ply[a]	Nil	7	30	<10	8	1	1	8.5×10^5	1.1×10^5	5	2	3
		Sterilized	7	30	<10	8	1	1	8.0×10^5	1.0×10	6	4	3
2.		Nil	14	<10	40	7	1	1	1.1×10^7	2.0×10^2	5	2	2
		Sterilized	14	<10	40	7	1	1	5.0×10^3	5.7×10^4	5	2	3
3.		Nil	21	10	<10	8	1	1	2.0×10^5	1.0×10^4	5	4	4
		Sterilized	21	10	<10	8	1	1	5.0×10^4	8.1×10^4	5	4	3
4.		Nil	24	<10	23	8	1	2	3.0×10^5	1.0×10^4	5	5	4
		Sterilized	24	<10	10	8	1	1	1.5×10^4	2.0×10^3	7	5	4
1.	4 ply[b]	Nil	7	30	<10	8	1	1	5.0×10^6	1.0×10^2	5	4	4
		Sterilized	7	30	<10	8	1	1	4.0×10^6	1.0×10^2	5	4	4
2.		Nil	7	<10	40	7	1	1	5.0×10^6	1.0×10^3	6	2	2
		Sterilized	7	<10	40	7	1	1	1.3×10^6	1.0×10^2	5	3	2
3.		Nil	14	10	<10	8	1	1	4.6×10^3	1.0×10^3	5	3	3
		Sterilized	14	10	<10	8	1	1	2.8×10^4	5.1×10^4	4	5	3
4.		Nil	14	<10	10	8	1	1	1.0×10^4	2.0×10^3	5	5	3
		Sterilized	14	<10	<10	8	1	1	9.0×10^4	2.0×10^3	5	5	3

a) Polyethylene/cellophane: *b*) Paper/polyethylene/foil/polyethylene.

and flavour (Table 3). The product was found acceptable in both cases of pouches even after 7 days' storage.

The samples of *khoa* packed in two types of pouches showed variable shelf life ranging from 7 to 22 days when stored at 37°C as also reported by Rudreshappa and De[12]. This can partially be attributed to variations in initial moisture contents (30-39 per cent) of *khoa* from different batches. However, the shelf life as well as storage patterns of *khoa* for a particular batch were identical irrespective of radiosterilization treatment given to the packages (Table 2 & 3).

Since the use of radio sterilized packages did not show any additional shelf stability to *khoa*, it may be concluded that thermophiles surviving heat processing of product and/or air contamination have significant role in affecting the keeping quality. Conversely, it may be inferred that packaging material does not contribute significantly to microbial contamination, because of plastic films already being processed at a temperature of about 110°C. Therefore, the possibility of microflora surviving in absence of any substrate are remote. However, incidence of high microbial contamination of the packages must not be ruled out if handled poorly. The product itself is not amenable to radiation treatment as it develops off-flavour and browning in it.

Acknowledgement

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IMPAIRED RENNET SUSCEPTIBILITY OF CASEIN MICELLES ON PHOTO-OXIDATION

Photo-oxidation of casein micelles from cow or buffalo milk reduced the release of sialic acid, glycomacropeptide, non-protein nitrogen and turbidity development in micelles by rennet. Micelles from cow milk exhibited higher susceptibility. Electrophoretic analysis and gel filtration data revealed aggregation of casein micelles due to photo-oxidation.

Casein and its fractions are susceptible to photo-oxidation[1,2]. Interaction of casein with calcium is markedly reduced[1]. Photo-oxidation of casein[2] and k-casein[3] involves oxidation of histidine and tryptophan residues. k-casein on such treatment loses its ability to stabilize s-casein and was not precipitated by rennin in the presence of calcium[3]. Hill and Laing[4] suggested that such change in the clotting property in k-casein occurs due to the alteration of histidine residue. In the present paper we report the changes in the casein micelles of cow and buffalo milk on photo-oxidation in relation to its rennet susceptibility, electrophoretic behaviour and molecular make-up.

Casein micelles were prepared from skim milk, cow or buffalo, by ultracentrifugation[5]. Casein micelles were photo-exidized in air in presence of methylene blue according to Hill and Laing[4].

Rennet was a preparation from Hansen Laboratory, Denmark. Sephadex G-100 was purchased from Pharmacia, Sweden while starch hydrolysate was from B.D.H. The releases of sialic acid, non-protein nitrogenn (NPN) and glycomacropeptides (GMP) from casein micelles by rennet were carried out according to Gupta and Ganguli,[6] Rowland,[7] and Banerjee and Ganguli,[8] respectively. Rennet action was also evaluated by the turbidimetric method of Sabarwal and Ganguli[9]. Starch gel electrophoresis of the micelles was performed according to Ganguli and Majumder[10]. For gel filtration studies with Sephadex G-100, the method of Yaguchi and Tarassuk[11] was followed.

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TABLE 1. RENNET SUSCEPTIBILITY OF THE CASEIN MICELLES AFTER PHOTO-OXIDATION*

Source of casein micelles	Released sialic acid (%)	Released NPN (% of total N)	Released GMP (Klett readings)
Buffalo			
a) Photo-oxidised	63.10 (61.74–64.54)	0.576 (0.504–0.600)	31 (30–32)
b) Control	75.49 (72.20–78.64)	0.753 (0.706–0.797)	51 (49–54)
Cow			
a) Photo-oxidised	55.43 (54.00–56.58)	0.894 (0.775–1.022)	106 (94–118)
b) Control	81.10 (78.31–84.13)	1.280 (1.190–1.363)	149 (134–159)

Figures within parentheses indicate range of variation
*The number of samples analysed in each case was five.

It is apparent from results in Table 1, that photo-oxidation of the casein micelles caused marked reduction in the release of sialic acid, NPN and GMP in both cow and buffalo milk. Cow micelles appear to be more susceptible. According to Hill and Laing,[4] the reduced NPN release is due to the modification of the histidine residue. The impaired release of sialic acid and GMP clearly indicates that k-casein is involved in the photo-oxidation process. Zittle[3] had shown that k-casein on photo-oxidation looses its ability to stabilize α-casein.

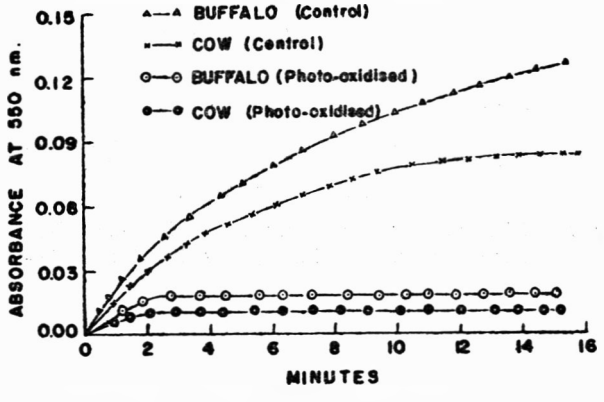

Fig. 1. Effect of photo-oxidation on the rate of turbidity development in casein micelles by rennet.

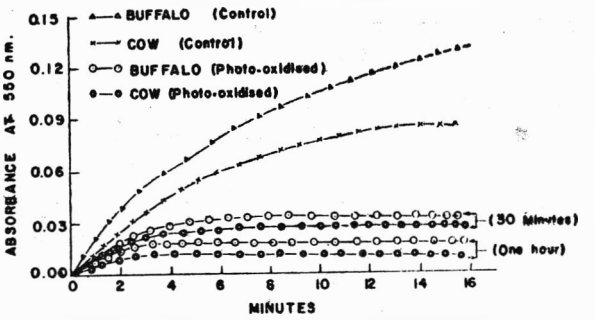

Fig. 2. Effect of photo-oxidation for limited periods (30 min, 60 min) on turbidity development in casein micelles by rennet.

Rennet action as assessed by the turbidity development in casein micelles,[9] clearly demonstrates that the photo-oxidized micelles are significantly less susceptible (Fig. 1) and the degree of susceptibility is dependent on the period of photo-oxidation (Fig. 2). In one hour exposure there was almost complete inhibition of rennet action. Thomas[12] reported that the methionine site of rennin action is exposed on photo-oxidation which is normally buried in a non-polar region. The liability of the rennin sensitive bond is much reduced by photo-oxidation of the methionine residue.

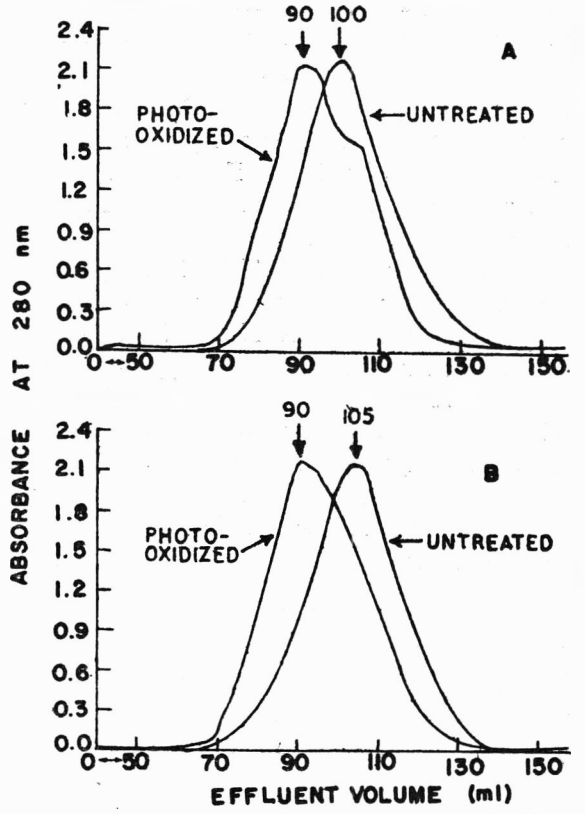

Fig. 3. Gel filtration of casein micelles on Sephadex G-100 as affected by photo-oxidation. A-Buffalo; B-Cow.

Photo-oxidation caused drastic change in the net charge of the micelles as a result of which the casein components did not move on the gel bed, under the conditions of electrophoresis[10]. This could be due to the aggregation of β-casein and k-casein on photo-oxidation as reported by Zittle[3]. The phenomenon of aggregation is further supported by the gel filtration profiles (Fig. 3) of the photo-oxidized casein micelles. Fresh casein micelles on photo-oxidation filtered out faster than the original micelles. In this specific case, both cow and buffalo micelles filtered out in the 90 ml-fraction instead of 100 or 105 ml-fraction (buffalo or cow micelles) for the untreated samples. This further supports that photo-oxidation catalysed the formation of larger micelles through the process of aggregation.

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BOOK REVIEWS

Animal Feeds from Waste Materials: By M. T. Gillies, Noyes Data Corporation, Park ridge, New Jersey, U.S.A., 1978. pp. 346, Price $ 39.

The book provides descriptive information on federally founded studies, conferences and U.S. patents issued since 1970, relating to production and manufacture of animal feeds from waste materials.

Waste disposal is becoming increasingly important and traditional disposal methods such as landfill, incineration and dumping into flowing water are turning to be less and less feasible with increased emphasis on air and water pollution problem by the regulating authorities, all over the World. The book covers the above subject and discusses the possibilities of converting an impressive percentage of waste into animal feeds.

The book contains six chapters. First chapter of the book describes processes that can be used for production of animal feeds using molasses, a by product of sugar industry and reduction of pollution problems arising out of molasses fermentation plants. The second chapter deals with production of animal feed from high cellulose wastes like bagasse, straw and wood. Utilisation of agricultural wastes like oil seed pulp, citrus pulp, beet pulp, waste potato products etc. forms the content of the third chapter. The fourth and fifth chapters of the book are devoted to utilisation of manure, sewage and other animal waste products. Utility of whey as an animal feed is the subject of sixth chapter.

The book is well organised and the information contained in it can be used to establish a sound background in the area of feed technology while taking up research programmes in the subject.

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Sensory Properties of Foods: G. G. Birch, J. G., Brennan and K. J. Parker, EDS., Applied Sciences Publishers Ltd., Ripple Road Barking, Essex, England 326+xi pp; 1977; $ 34.

The developments in food technology, arising out of the demand for convenience foods and the anticipated shortage of traditional foods such as meat and other animal foods, have brought sharply into focus the sensory qualities of foods which form the basis on which the consumer makes his choice. This industrial interest has led to much research aimed at acquiring a basic understanding of the objective parameters of the final food products, and their correlation with the subjectively appreciated attributes such as colour and appearance, taste, aroma, and texture (including mouthfeel). These aspects are discussed in four sessions, through comprehensive reviews on basic aspects such as measurement of colour, volatiles, and texture, relation of structure of stimuli (food components) and chemoreception, correlation of objective and subjective measures of food aroma and texture, new uses of the subjective measuring technique of magnitude estimation, and the factors influencing the sensory attributes of a few specific commodities.

The reviews are by active workers from England, Europe and America who are well known in their respective fields of work. The happy blend of authoritative reviews on structure and perception, and on modification of tastes, indicating future possibilities and on practical approaches to the subjective measurement of the aroma, taste, and texture of foods, and the objective measurement of the corresponding stimuli and rheological properties with the object of predicting acceptability to the consumer must have been an exhilarating experience to the participants from industry in the industry-university cooperation symposium organised by the University College of food Technology, University of Reading. For others who are familiar with the large volume of recent literature in these areas, the publication will serve as an excellent status report. There is little new information presented, which has probably made the discussions that followed, rather ordinary. An interesting point made is that the sensory attributes are descriptions of the reactions to the stimuli provided by the food. So, does food have sensory properties?

A theme running through all the reviews is the need to understand better the way the human senses integrate and take a total view of the different food attributes, without necessrily measuring the significant individual food properties or components. Modern instrumentation and computerized analysis are extremely efficient in separation and quantitation, but what needs to be determined is the significant interactions of components contributing to the sensorily perceived attributes, and the interplay of different attributes which results in acceptance or rejection. Besides, there is the large area of subjective reactions to food which gives meaning to what is perceived as quality of food. There is much to be learnt in this area of psychometry and relating it to

consumer decision. The reviewer feels that a review of factors influencing the consumers' choice of food and methods of studying such factors would have added much value to the publication.

The publication is excellently edited and produced and is a pleasure to read. This will be a welcome addition to all industrial and academic (university) laboratories, concerned with food product development and evaluation.

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Perspective in Industrial Microbiology: Proceedings of the Symposium held at Hindustan Lever Research Centre, Bombay (2nd and 3rd April 1977), Eds. G. P. Kalle, Yvonne M. Freitas and D. V. Tamhane, Published by the Association of Microbiologists of India (Bombay Unit), 1978 Rs. 20/-.

The book is a publication of the proceedings of a symposium on the perspectives in industrial microbiology with special reference to India. According to the organisers, "The major purpose of the symposium was to bring about a greater awareness among scientists, working on such esoteric problems facing the industrial microbiologists in India today; and to make technologists conscious of the need to derive the relevant benefits of research carried out in Indian laboratories".

The symposium topic is covered in six chapters. (1) Perspectives, (2) Fermentation as low cost technology, (3) Chemicals through fermentation, (4) Immobilization as a technique for fermentation, (5) Application of genetics to industrial fermentations, and finally (6) A guest lecture on the Indian scene on fermentation technology. The first, much of the third, part of the fourth and the final chapters indicate extensive possibilities (in India) for the fermentation industries—from the expensive vitamin B-12, gibberellic acid and steroids to cellulose fermentation including gobar gas production and single cell proteins. These are competent reviews. It is heartening to learn that processes for production of lysine and citric acid are ready and lactic acid is being commercially produced in India.

The work on gobar gas production is highly commendable. The authors have persevered in this work for many years and have made extensive meaningful studies on fermentation conditions, yields, design of the digester and engines which can use the gas. Similarly the work on cellulose utilization is significant and it is hoped that soon the process can be scaled up and its preliminary practicability tested. The review on immobilization of enzymes and microbial cells is good but one would have liked to know in some detail the present status of such work (e.g. production of 6-amino-penicillanic acid) done and prospects in India. The work on liposomes and their possible medical use is thought-provoking and one hopes that these will work out an effective and specific (drug) delivery system for specific tissues. However, getting this topic into an industrial microbiology symposium is also a good innovation. The chapters on the application of genetics are good short reviews.

The book contains much useful information regarding industrial fermentations/microbiology. It would have been more relevant if recommendations had been made regarding-specific priorities based on national needs, personnel and technological capabilities and limitations, finances, etc.—for the next 5 to 10 years. No other country can serve as a model for us-neither Japan with its multitudinous, traditional, microbiologically derived/processed foods, nor the USA with its vast agricultural surpluses and wastes, although there may be a few common points. While the objectives of the symposium are unexceptionable, it is hoped that their discussion and dissemination has been useful.

The get up of the books is attractive and one gets a good and satisfying bargain for the price.

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Foods, Drugs and Cosmetics: edited by Thankamma Jacob, Published by the Macmillan Co. of India Ltd, 6 Pathulos Road, Madras-600 002, 1977, Pages 169, price Rs. 12/-.

There is, as yet, no strong consumer movement in our country. This is perhaps partly due to apathy and partly due to ignorance of their rights. The book "Foods, Drugs and Cosmetics" may be expected to play a useful role in educating the consumers on the possible undesirable effects of some articles of importance in every day life.

The book deals with several diverse aspects, such as food adulteration, food habits, environmental safety and hygiene, drugs and cosmetics—a rather assorted group of topics. Drawing attention to the existing Prevention of Food Adulteration Rules and stating them in simple terms, as also providing information as to whom to report violation of the law, avoidance of loopholes in the law etc. would have been helpful to consumers. The

book contains some statements which would have better been avoided. For example, it is stated that "it is virtually impossible for the average consumer to find out the presence of moulds....." (p. 34). In the very next page, it is stated that "if mould growth is noticed on any food.....", most people can detect mould growth and mould damage.

The validity of some statements is open to question. For example, it has been said that "food fortification with iron on a large scale is definitely not a practicable proposition, nor a correct approach.....". (p. 54). That is practicable has been shown, and that it is not a correct approach debatable.

Despite these criticisms, this paper back is informative and well produced. Since the book is meant primarily for the lay consumer it is neither scholarly in style nor academic in nature, but is a useful and readable book for those interested in the consumer movement. Although in some places there is a tendency for repetition, this book is a good companion to the earlier volume by the same author.

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Handbook on Package Materials: Edited by Sacharow, Published by AVI Publishing Co., Connecticut, USA, 1976, Pages 240, Price U.S. $ 23.

The publication can serve as a useful compendium for the students who wish to study the subject of packaging materials, their properties and to some extent their compatability for various types of products. The treatment of the subject is no doubt informative but needs to provide guidelines to the students in matters of choice of one type of material vis-a-vis the other. Packaging design, styles, shapes are integral parts of packaging development in relation to the environmental parameters obtaining in a particular set up. These have to be linked up with conditions of storage, handling, transportation, distribution practices and consumer's choices. In addition, the choice of material is very much conditioned by the development of technology and the existence of a high level of packaging consciousness. It would have been proper if of chapters had been devoted to the appropriateness of technology and inter-se merits and demerits of the packaging materials with due emphasis on certain illustrations from the industrial field. The reader would have felt greatly benefitted from the illustrations and derived his own conclusion regarding the suitability of different products. Similarly, a mention about methodology for material characteristic testing can infuse the desire and help to maintain quality standards and improvements.

To this extent, the treatment of the subject has left a gap to be abridged.

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BOMBAY-93.

Dietary Fats and Oils in Human Nutrition: Report of U. N., Published by Food & Agriculture Organisation of U. N., Rome Italy, 1977, pp. 94.

The report brought out by an expert consultation group jointly organised by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) held in Rome, September 1977, contains a wealth of information of current interest in the area of lipids in human nutrition. The group was well represented by authorities who had already made excellent contributions in the area.

The report is divided into well compiled chapters based on fat as a source of energy, the use of fat in adult and child feeding, essential fatty acids and prostaglandins, essential fatty acids in early development, fat in the prevention and treatment of cardiovascular diseases, obesity and diabetus mellitus. In addition, considerable importance is also given to effects of processing on the nutritive value of fats and oils in human nutrition as well as health implication on consumption of Brassica-derived oils rich in erucic acid. Long-chain fatty acids in marine oils and uneven fatty acids have been also discussed. Finally, recent trends in production, trade and consumption of fats and oils and the impact of plant and animal breeding and management on quality and composition of fats and oils have been elucidated. As with most meetings with motivated group of research scientists, a brief and well compiled recommendations of the meeting have been highlighted followed in appendices by fatty acid content of some foods and home-made preparations as supplementary foods.

The first few chapters deal essentially with functions of dietary fat and the important problem faced in many developing countries of protein-calorie malnutrition in infants and in pregnancy and lactation. A good account is rendered on the metabolism and essential fatty acid deficiency syndrome in humans and linolenic acid as an essential fatty acid and adverse effects of polyunsaturated fats in nutrition. Cardiovascular diseases, obesity, diabetus mellitus, arterial thrombosis and hypertention have been discussed adequately as pro-

blems affecting mainly the developed countries and how judicious use of lipids can go a long way in controlling such maladies.

The presence of erucic acid in oils derived from seeds of Brassica, e.g., rapeseed and mustard oils and how consumption of such oils on laboratory animals caused growth retardation and changes in the heart, adrenals and liver is discussed. In recent years there is evidence of such effects in human population as well. A well-compiled table is presented showing the adverse effects on several organs of different species of animals on consumption of rapeseed oil rich in erucic acid. Similarly, the adverse effects on consumption of marine oils have been high-lighted. The chapter on recommendations should prove most useful to scientists who are interested in adequate planning of dietary consumption of lipids with respect to human nutrition.

On the whole, the broad-based report on lipids is a very useful compilation of deliberations by authorities in the field and should prove a very useful report.

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Lipids and Lipid Polymers in Higher Plants: Ed: M. Tevini and H. K. Lichtenthaler, Springer-Verlag Berlin, Heidelberg, New York, 1977. XIII+306 PP. Price U.S. $ 39.60.

A symposium on "Lipids and Lipid Polymers in Higher Plants" was held in July 1976 at the Botanical Institute of the University of Karlsruhe with the intention of bringing together some of the leading Scientists in various areas of contemporary research in plant lipids. A variety of topics ranging from fatty acids to lipid polymers were discussed with emphasis on their distribution, biosynthesis and physiologic function in biological membranes. The main topics discussed at this symposium have been elegantly presented in this book.

The book contains 16 articles under 5 sections. The first is devoted to functional organization of biomembranes with articles on prenyllipids, phospholipids and glycolipids of higher plant membranes. The second section deals with physiology and biochemistry of fatty acids and glycerides, with articles on lipid biosynthesis in developing seeds, Cycloprane fatty acids, galactolipid biosynthesis and role of lipids in plastid development. Biosynthesis and physiology of plant sterols and stearyl glycosides comprise the next section. The fourth section contains articles on the biological significance of prenols and their phosphorylated derivatives, carotenoids in higher plants, regulation of prenylquinone synthesis and chlorophyll formation. In the final section, biosynthesis, degradation and intermolecular structure of plant lipid polymers such as cutin and suberin have been reviewed.

The articles cover almost the entire field of plant lipids but highlight mainly the significant findings and progress made in the recent past. The topics were discussed authoritatively and the lacunae in our knowledge have been sharply pointed out. The editors have assembled a volume that is purposeful, exhaustive and timely. The book would be very useful both for the advanced student and research worker in plant lipid biochemistry for a long time.

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ASSOCIATION NEWS

Trivandrum Chapter:

The Annual General Body Meeting of the Chapter was held on June 10, 1978 at Trivandrum Club. Mr. C. J. Philip, the President of the Chapter welcomed the members. Mr. A. George Varkey, Hon. Secretary read the report on the activities of the Chapter during 1977–78 Mrs. Emilia Abraham, Hon. Treasurer presented the Statement of Accounts.

Following Office-bearers were elected for 1978–79.

President

Mr. R. Hariharan, M/s Kirthika Agencies, East Fort, Trivandrum.

Hon. Secretary

Mr. A. V. Bhat, Scientist, CSIR Complex Trivandrum.

Hon. Treasurer

Mr. C. Balachandran, Scientist, CSIR Complex, Trivandrum.

After the induction of the new President, the meeting ended with a vote of thanks by Mr. A. George Varkey, the outgoing Hon. Secretary of the Chapter.

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ANNOUNCEMENT OF

SUMAN FOOD CONSULTANTS TRAVEL AWARD 1978-79

The Association of Food Scientists and Technologists (India), have instituted a Travel Award in the name of "Suman Food Consultants" to Post-graduate Degree/Diploma students in Food Science/Technology. The award will be of Rs. 300/- (Three Hundred only), which will enable the Awardee to attend the Annual General Body Meeting and the Technical Seminar/Symposium of the AFST in that year.

The selection of the Award will be based on an Essay Competition on the following topic:

Role of Food Additives in Food Processing and Public Health

The Essay should normally not exceed 20 thesis-size typed pages. This may be forwarded to the undersigned in QUADRUPLICATE with complete bibliography on or before 1st January 1979 for onward transmission to the AFST.

The best Essay will be selected by a Panel of Experts constituted for this purpose by the Executive Committee of the Association and the Awardee informed by the selection by the first week of February 1979. The Annual General Bond Meeting is proposed to be held some time in 1979.

Sd/ **S. P. MANJREKAR**
Convenor
Suman Food Consultants Travel Award Committee

ANNOUNCEMENT

OF

Prof. V. Subrahmanyan Industrial Achievement Award for the Year 1978

Nominations for the above award for the year 1978 are indicated. The guidelines for the award are as follows:

1. Only Indian Nationals engaged in the field of Food Science and Technology will be considered for the award.

2. The nominee should have contributed to the field of Food Science and Technology, for the development of Agro-based food and allied industries or to basic food science and technology with immediate prospects and/or future potential for industrial application.

3. The Nominee should be proposed by any member of the Association; the bio-data of the candidate should be given in detail including the work done by him and for which he is to be considered for the award.

4. The awardee will be selected (from the names sponsored) by an Expert Panel constituted by the Executive committee for the above purpose.

Nominations along with bio-data and contributions, should be sent by **Registered Post** *so as to reach* **Dr. J. V. Prabhakar,** *Honorary Executive Secretary, Association of Food Scientists and Technologists (India), Central Food Technological Research Institute, Mysore- 570 013 latest by 31st of January 1979.*

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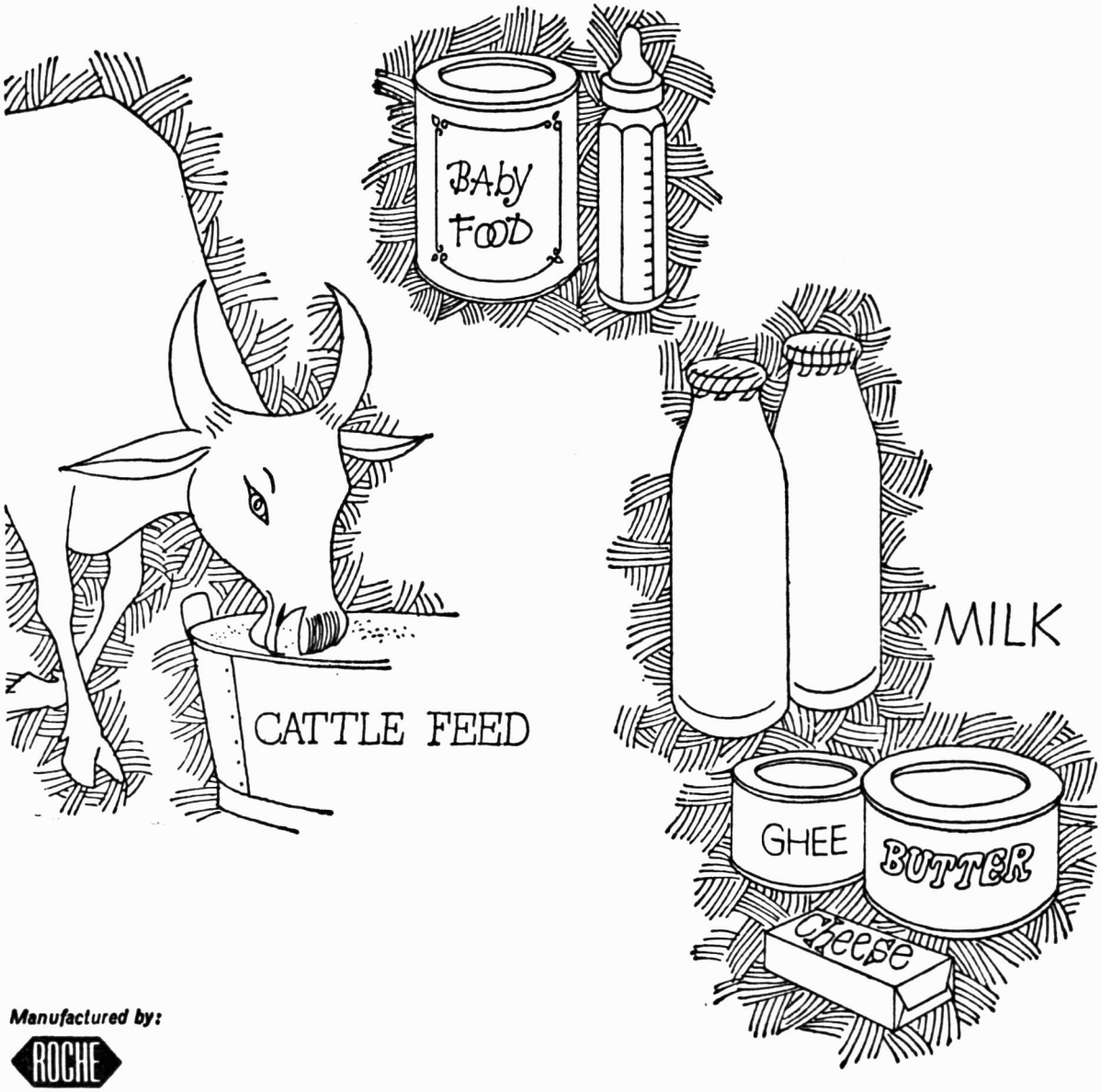

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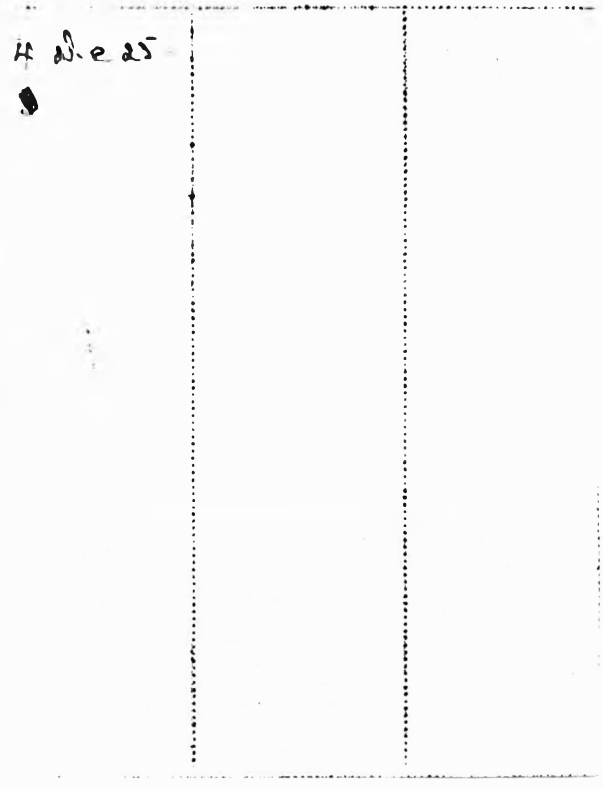

INSTRUCTIONS TO CONTRIBUTORS

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

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

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

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

5. **Tables:** Graphs as well as tables, both representing the same set of data, should be avoided. Tables and figures should be numbered consecutively in Arabic numerals and should have brief titles. Nil results should be indicated and distinguished clearly from absence of data.

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

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

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

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

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

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

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

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

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

   (*e*) *Thesis:* Sathyanarayan, Y., Phytosociological Studies on the Calcicolous Plants of Bombay, 1953, Ph.D. thesis, Bombay University.

   (*f*) *Unpublished Work:* Rao, G., unpublished, Central Food Technological Research Institute, Mysore, India.

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