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Iron and Its Availability in Maize (*Zea Mays* L.)

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The study was undertaken to determine the status of iron in 'Opaque-2' (both soft and hard endosperm types which are known to be high in mineral, lysine and tryptophan contents) and normal maize varieties after processing. Total iron content in 'Ganga-5' (normal endosperm), SO/SN composite (hard endosperm type) and 'Shakti' composite (soft endosperm) kernels was 2.34, 5.72 and 7.70 mg/100 g, and the soluble fraction being 66.7, 68.01 and 75.1% respectively. Removal of pericarp slightly decreased the iron content. Iron content in any of the three varieties was not reduced on boiling. Estimates of iron availability in these three varieties followed a pattern similar to those for soluble iron.

Iron deficiency is a common nutritional deficiency in developing countries. Fibre is reported to interfere with iron absorption due to its binding capacity¹. High consumption of fibre from maize tortilla may affect zinc absorption and incorporation of maize into diet decreased iron absorption². A study was undertaken to determine the effect of pericarp on iron availability in 'Opaque-2' and normal maize varieties with and without processing, as pericarp fraction of the whole kernel is the richest source of cellulose and hemicellulose³.

Materials and Methods

The present investigations were carried out on three maize varieties, namely 'Ganga-5' (normal), 'SO/SN composite' (hard endosperm 'Opaque-2') and 'Shakti' (chalky opaque-2) grown at the Institute farm during monsoon season of 1985. The self-pollinated ears were harvested at maturity. In each of the three varieties, kernels were divided into two equal portions. One portion was boiled for 40-45 min in double distilled water (1:3 W/V) and then dried in a lyophilizer and the other portion was used as such. The pericarp of half of the kernels of each portion was removed.

Soluble iron extraction was done according to the method of Narasinga Rao and Prabhavathi⁴ with slight modification using 0.5 per cent pepsin (from Merck, 70 FP U/g) - HCl solution (pH 1.35) and followed by digestion of dried extract with tri acid mixture (10:1:4, HNO₃ : H₂SO₄ : HClO₄ V/V) according to Piper⁵. For total iron, the sample was digested with tri acid mixture and estimation was done colorimetrically according to Toth *et al*⁶.

The *in vitro* iron availability from the dried samples was estimated according to the procedure of Gupta *et al*⁷ using pronase (from Koch-Light) and trypsin (from Sigma) with the addition of 0.5 per cent pepsin (from Merck, 70 FP U/g). Iron content was determined separately in both, supernatant and residue.

$$\text{In vitro iron availability in ppt} = \frac{\text{Total Fe in sample} - \text{Total Fe in ppt}}{\text{Total Fe in sample}} \times 100$$

$$\text{In vitro iron availability in supernatant} = \frac{\text{Total Fe in supernatant}}{\text{Total Fe in sample}} \times 100$$

Means of both values were used.

Neutral detergent fibre (NDF) was determined in fresh as well as boiled kernel samples with and without pericarp by the method of Goering and Van Soest⁸.

Results and Discussion

Neutral detergent fibre: In fresh and boiled whole kernels with and without pericarp, NDF was highest in chalky 'Opaque-2', followed by hard endosperm 'Opaque-2' and was least in normal kernels (Table 1). This trend was the reverse in the case of pericarp alone. After removal of pericarp in either fresh or boiled kernels, NDF decreased in all the varieties. After boiling, NDF increased 11.0, 8.0 and 6.5 per cent in normal, hard endosperm opaque-2 and chalky 'Opaque-2' kernels, respectively with pericarp whereas without pericarp, the increase was marginal.

Total, soluble and insoluble iron: In general,

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TABLE 1. NEUTRAL DETERGENT FIBRE (NDF) (G/100 G) IN MAIZE

Variety	Pericarp only	Fresh kernel		Boiled kernel	
		With pericarp	Without pericarp	With pericarp	Without pericarp
Ganga 5	87.40	15.50	9.40	17.20	9.60
SO/SN composite	86.65	16.80	10.20	18.14	10.35
Shakti	84.90	32.54	20.54	34.64	21.05
Means of triplicate analysis					

TABLE 2. TOTAL AND SOLUBLE IRON (MG/100 G) IN MAIZE

Variety	Type of Fe	Fresh kernel		Boiled kernel	
		With pericarp	Without pericarp	With pericarp	Without pericarp
Ganga 5	Total	2.34	1.87	2.36	1.86
	Soluble	1.56 (66.70)	1.21 (64.70)	1.52 (64.40)	1.30 (69.90)
SO/SN composite	Total	5.72	5.23	5.83	4.99
	Soluble	3.89 (68.01)	3.42 (65.39)	3.88 (66.60)	3.54 (70.90)
Shakti	Total	7.70	5.72	7.75	5.70
	Soluble	5.78 (75.10)	4.09 (71.50)	5.59 (72.10)	4.34 (76.10)

Means of duplicate analysis

Figures in parentheses indicate per cent of total iron

chalky and hard endosperm 'Opaque-2' kernels contained higher total iron than normal kernels (Table 2). After removal of pericarp, its concentration decreased in both fresh and boiled kernels of all the varieties. Marginal increase and decrease in total iron were observed in kernels having pericarp and kernels without pericarp respectively on boiling. Soluble iron is the major fraction of the total iron in all the maize varieties. Its concentration was also higher in chalky 'Opaque-2', followed by hard endosperm 'Opaque-2' and least in normal kernels in all the treatments. After removal of pericarp, soluble iron on the basis of mg/100 g also decreased in both fresh and boiled kernels whereas on the basis of per cent of total iron, it decreased in fresh kernels and increased in boiled kernels of all the varieties. Similar and reverse trends were observed for insoluble iron respectively as mg/100 g and as per cent of total iron in all the varieties and treatments as it was calculated by subtracting soluble iron from total iron.

In vitro iron availability: *In vitro* iron availability was also higher in chalky and hard endosperm, 'Opaque-2' kernels of all the four treatments compared with their normal kernels respectively (Table 3). In fresh kernels of chalky and hard endosperm 'Opaque-2', *in vitro* Fe availability was

TABLE 3. *IN VITRO* AVAILABILITY (PER CENT) OF IRON IN MAIZE

Variety	Fresh kernel		Boiled kernel	
	With pericarp	Without pericarp	With pericarp	Without pericarp
Ganga 5	55.17	43.75	44.95	61.91
SO/SN composite	58.10	50.81	51.98	62.55
Shakti	66.64	50.63	52.58	70.11
		Varieties	Treatments	
SEm		±0.60	±0.69	
CD at 5%		1.71	1.98	
Means of duplicate analysis				

11.47 and 2.93 per cent higher with pericarp and 6.88 and 7.06 per cent higher without pericarp whereas in boiled kernels, its value was 7.63 and 7.03 per cent higher with pericarp and 8.20 and 0.64 per cent higher without pericarp, respectively. After removal of pericarp, *in vitro* iron availability decreased in fresh kernels and increased in boiled kernels in all the test varieties.

Pericarp, which is the richest fraction of dietary fibre in the maize kernels⁹, contained more than 84 per cent NDF. Schaller¹⁰ has also reported similar results for maize bran. Pericarp fraction in mature maize kernels was reported to be 7 per cent in 'Opaque-2' and 9.3 per cent in normal³. *In vitro* iron availability and soluble iron per cent decreased in fresh kernels and increased in boiled kernels after removal of pericarp. This is due to the fact that fibre has iron binding capacity¹. This property of fibre – iron interaction becomes more effective in the boiled kernels with pericarp (Maillard reaction). Highly significant positive correlation ($r = 0.8097$) between per cent of soluble iron and negative correlation ($r = -0.8088$) between per cent of insoluble iron with *in vitro* iron availability support the above findings.

After boiling, increase in NDF is attributed to the production of insoluble proteins and other non-digestible products which are produced by Maillard reaction during cooking¹¹. At the same time, boiling also causes breakdown of hydrogen bonds and release of certain nutrients in the free form by hydrolysis of peptide bonds¹². Inorganic iron from maize kernels which is poorly absorbed compared with haem iron from meat, may increase significantly after removal of pericarp of boiled kernels.

Therefore, consumption of boiled maize kernels without pericarp can partly combat iron deficiency problem in the population where animal products form a very small part of diet. Utilization of chalky and hard endosperm 'Opaque-2' in this way will be more nutritious as these varieties have not only better quality of protein but are rich in minerals also¹³.

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Studies on the Development of Methods for Production of Quick Cooking Rice

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To produce quick cooking dehydrated rice with short reconstitution time, rice was cooked by boiling for 25 min, partially boiled for 10 min and steamed at 1.1 kg/cm² for 5 min or steamed alone for 10 min at 1.1 kg/cm² followed by tray drying (both atmospheric and vacuum), infra red drying, air diffusion drying and freeze drying. Atmospheric tray drying produced quick cooking rice of inferior quality having low swelling ratio (by weight) of 3.0-3.6 and long reconstitution time of 10-14 min; the retention of vitamins B₁ and B₂ was also low. In vacuum tray drying, infra red drying and air diffusion drying, the swelling ratios were 3.1-3.7, 3.2-3.7 and 3.4-3.6 with reconstitution times of 9-12, 9-12 and 9-10 min, respectively. These also showed higher retention of vitamins B₁ and B₂. Highest swelling ratio of 3.8 with a reconstitution time of 7 min was observed in the freeze dried product; retention of vitamin B₁ and B₂ was also highest in this.

Parboiled rice normally requires cooking for 20 to 30 min. Precooked and dried (quick cooking) rice as a convenience food is available in many countries, which can be reconstituted in 2 to 5 min of cooking. The process for the manufacture of quick cooking rice generally involves soaking, cooking and drying of the rice. Certain other treatments are necessary to facilitate hydration and gelatinisation. Different patented processes have been described¹. Instant rice was also prepared by roasting followed by precooking and dehydration in a cross flow tray dryer or in a fluid bed dryer. Pre-treatment with chemicals, however, did not show any beneficial effect in reducing the reconstitution time^{2,3}. Chemical treatment followed by freeze drying upto 20 per cent moisture and subsequent air drying upto 12 per cent moisture was tried where the retention of nutritional value was maximum⁴. The present investigation was undertaken to develop improved methods for production of quick cooking rice and evaluate the comparative quality of the products obtained by different combinations of cooking and drying.

Materials and Methods

Milled and cleaned long grain "Patnai" parboiled rice (10.3 per cent moisture) procured from local wholesale market was used in the investigations. Parboiled rice being non-sticky was selected as it can be handled conveniently. The head rice was separated from broken manually. Optimal cooking time was determined by the method of Chakrabarty *et al*⁵.

The following cooking methods were adopted.

i) *Cooking by boiling*: Rice was soaked in twice its

weight of water for 30 min at room temperature (approximately 25°C). After draining the excess water, the soaked rice was cooked in 6 times its weight of water for 25 min. Cooking loss in g-ruel was estimated from the excess water drained after cooking and by removing the adhering water on the rice with the help of blotting paper. Both the drained water collected in a beaker and the blotting paper were dried and weighed. The increase in weight of the beaker and the blotting paper indicated cooking loss.

ii) *Cooking by partial boiling followed by steaming*: Rice was soaked as before and cooked in 6 times its weight of water for 10 min. Excess water was collected as before. Partially cooked rice was steamed by autoclaving at 1.1 kg/cm² for 5 min. Cooking loss was determined as before.

iii) *Cooking by steaming*: Rice was soaked as before. The soaked rice was steamed by autoclaving at 1.1 kg/cm² for 10 min. The adhering water was removed and the cooking loss calculated.

The following drying techniques were adopted. One hundred grams of cooked rice obtained from each method of cooking were dried in the following types of dryers.

i) *Tray drying*: An electrically heated cross flow type dryer with perforated trays having sizes 30 cm × 30 cm × 15 cm was used. The trays were arranged in two shelves each containing 4 trays placed one below the other. The air flow rate was kept constant at 7.1 m³/min; the temperature and RH of air were 65°-100°C and 5-10 per cent respectively. During the drying cycle, the material inside the dryer was taken out at intervals of 30 min and kept in a closed vessel

for 5 min for conditioning (to ensure equilibration of moisture in order to reduce drying time). The process was repeated 2-4 times for samples depending on the temperature and time of drying. After drying, the moisture contents were determined for all the samples, which were then packed and sealed in 300 gauge polyethylene bags and stored for further study.

ii) *Vacuum tray drying*: An electrically heated cabinet type dryer with two fixed plates and the chamber connected to a vacuum pump was used. The cooked rice was spread over a 25 mesh ISS brass wire screen and placed on the plate and dried at 60° and 100°C under vacuum (750 mm Hg).

iii) *Infra-red drying*: The cooked rice was spread over a porcelain plate and placed under the infra-red lamp (250 W) of the dryer (local make). Air was passed from a blower through a duct (15.8 cm × 9.6 cm cross section) at air flow rate of 0.71 m³/min over the rice. The temperature of the exit air was 38°C.

iv) *Air diffusion drying*: Air dehumidified by passing through a 60 cm high silica gel column was allowed to diffuse through a silk membrane (30 mesh ISS) upon which the cooked rice was placed in a vertical glass of 7.6 cm diameter. The material was dried at an air flow rate of 0.008 m³/min and a temperature of 26°C having 5 per cent RH.

v) *Freeze-drying*: Freezing and drying were carried out in two separate units. The cooked rice was frozen at -20°C for 2 hr in a deep freeze. The frozen material was immediately placed in a laboratory drying unit (local make) and operated at a pressure of 0.02 mm Hg and temperature -30°C. The time required for complete drying was 20-24 hr for different batches.

vi) *Freezing, thawing and drying*: This modified process consists of freezing the cooked rice at -20°C for 2 hr in a deep freeze and thawing at room temperature (25°C) for 2 hr followed by drying in a tray dryer (both atmospheric and vacuum) and infra red dryer.

Swelling ratio: This was determined by boiling a 10 g batch of dehydrated rice in 60 ml distilled water. At different intervals, samples were withdrawn and the weights of the samples were determined after removing the surface moisture with blotting paper. The swelling ratio (by weight) was expressed as the ratio of final to initial weight of the sample. The time required for complete rehydration was noted when there was no further increase in the final weight of the sample during cooking (all data are on dry basis).

Reconstitution time: Reconstitution time was noted as the time when the dehydrated rice samples attained the maximum swelling ratio (by weight) during cooking by boiling.

Chemical analysis: Moisture content was

determined by the method of AOAC⁶ and expressed on dry weight basis. The rice samples were ground to 30-40 mesh before moisture determination. Vitamins B₁ and B₂ were determined on dry basis by the thiochrome and fluorometric methods respectively⁷.

Results and Discussion

Table 1 summarises the time for drying of rice cooked under different conditions and dried in different dryers. The cooking loss was 2.8 per cent when the soaked rice was partially boiled and steamed and 0.8 per cent when cooked by steaming only as compared to 4.3 per cent when cooked in boiling water. The time for reducing the moisture from 73.1 to 9.8 per cent in a tray dryer was 2.75 hr when the temperature was maintained at 65°C. The drying time was reduced to 1.33 hr when the temperature was raised to 100°C. However, when the samples were frozen and thawed before drying in the tray dryer, the drying time was found to be reduced to 2.33 hr when dried at 65°C. Freezing and thawing might have hardened the capillaries which helped in lowering the drying time. The drying time for samples cooked fully or partially with steam was relatively less mainly because of lower moisture in the cooked samples. The results further established that the time of drying was minimised when drying was carried out under vacuum. In case of infra red drying, the drying time was considerably reduced because of penetration of heat to the interior of rice. In case of air diffusion drying and freeze drying, the drying time was considerably high, as the temperature was relatively lower. The experiments indicated that the methods of cooking influenced the quality of the finished products. When the samples were cooked by partial boiling followed by steaming or by steaming alone, the swelling ratio decreased irrespective of the methods of drying (Table 1). This may be due to excessive gelatinisation in the steaming process. But in samples frozen and thawed after cooking in boiling water, and dried in any dryer the swelling ratio was found to be relatively high compared to other samples. The swelling ratio in such cases was between 3.6 and 3.7 while those of freeze dried and control samples were 3.6 and 3.7 respectively. It was further observed that drying at higher temperatures (80-100°C) (both atmospheric and vacuum) produced products of better swelling ratio compared to those dried at lower temperature, which may be due to the development of excessive cracks on the grain at higher temperatures. Whatever was the method of drying, the reconstitution time of quick cooking rice was found to be reduced when freezing and thawing were done after cooking the rice in boiling water. But the lowest reconstitution time of

TABLE 1. CHANGES IN MOISTURE CONTENT OF COOKED AND DRIED RICE AND THE NUTRITIONAL VALUE, RECONSTITUTION TIME AND SWELLING RATIO OF QUICK COOKING RICE

Cooking method	Conditioning time (min)	Moisture in cooked rice (%)	Cooking loss (%)	Drying (temp) (°C)	Drying time (hr)	Final moisture (%)	Swelling ratio	Reconstitution time (min)	Vitamins (µg/g) (DB)	
									B ₁	B ₂
<i>Tray dried</i>										
Boiling	20	73.1	4.3	65	2.75	9.8	3.3	13	0.94	0.14
Boiling	15	73.1	4.3	80	2.00	9.6	3.4	12	0.91	0.11
Boiling	10	73.1	4.3	100	1.33	8.7	3.5	12	0.84	0.09
Part boil, + Steam	20	60.2	2.8	65	2.17	9.2	3.1	14	0.96	0.15
Part boil, + Steam	10	60.2	2.8	80	1.50	9.2	3.2	14	0.92	0.13
Part boil, + Steam	10	60.2	2.8	100	1.25	9.1	3.4	15	0.88	0.12
Steaming	15	51.8	0.8	65	1.83	9.0	3.0	14	0.98	0.15
Steaming	10	51.8	0.8	80	1.33	9.0	3.1	14	0.92	0.13
Steaming	5	51.8	0.8	100	1.00	8.8	3.2	15	0.90	0.13
<i>Frozen, thawed and tray dried</i>										
Boiling	20	73.1	4.3	65	2.33	9.3	3.6	10	0.95	0.15
<i>Vacuum tray dried</i>										
Boiling		73.1	4.3	60	1.70	9.2	3.4	11	1.36	0.16
Boiling		73.1	4.3	100	1.00	9.0	3.6	11	1.31	0.17
Part boil, + Steam		60.2	2.8	60	1.00	8.2	3.2	12	1.38	0.17
Part boil, + Steam		60.2	2.8	100	0.75	8.0	3.4	12	1.32	0.16
Steaming		51.8	0.8	60	0.83	8.1	3.1	12	1.40	0.17
Steaming		51.8	0.8	100	0.58	8.9	3.4	12	1.34	0.16
<i>Frozen, thawed and vacuum tray dried</i>										
Boiling	-	73.1	4.3	60	1.33	8.4	3.7	9	1.37	0.17
<i>Infra red dried</i>										
Boiling	-	73.1	4.3	38	1.00	8.2	3.5	10	1.41	0.17
Part boil, + Steam	-	60.2	2.8	38	0.67	8.0	3.3	12	1.44	0.18
Steaming	-	51.8	0.8	38	0.63	7.9	3.2	12	1.47	0.18
<i>Frozen, thawed, and infra red dried</i>										
Boiling	-	73.1	4.3	38	0.83	8.1	3.7	9	1.42	0.18
<i>Air diffusion dried</i>										
Boiling	-	73.1	4.3	26	42.00	10.4	3.6	9	1.42	0.17
Part boil, + Steam	-	60.2	2.8	26	40.00	10.2	3.4	10	1.44	0.18
Steaming	-	51.8	0.8	26	38.00	10.1	3.4	10	1.49	0.19
<i>Freeze dried</i>										
Boiling	-	73.1	4.3	-30	24.0	6.8	3.8	7	1.72	0.23
Part boil, + Steam	-	60.2	2.8	-30	20.0	6.2	3.6	8	1.78	0.23
Steaming	-	51.8	0.8	-30	20.0	6.0	3.6	8	1.80	0.25
Control Parboiled rice	-	-	-	-	-	-	3.7	25	2.32	0.32

7 min was observed in freeze dried samples. When rice was cooked in boiling water, vitamin retention was less in comparison with the other two methods of

cooking (Table 1). The temperature of drying also influenced the losses of vitamins B₁ and B₂; losses of vitamins were more at higher drying temperature. The

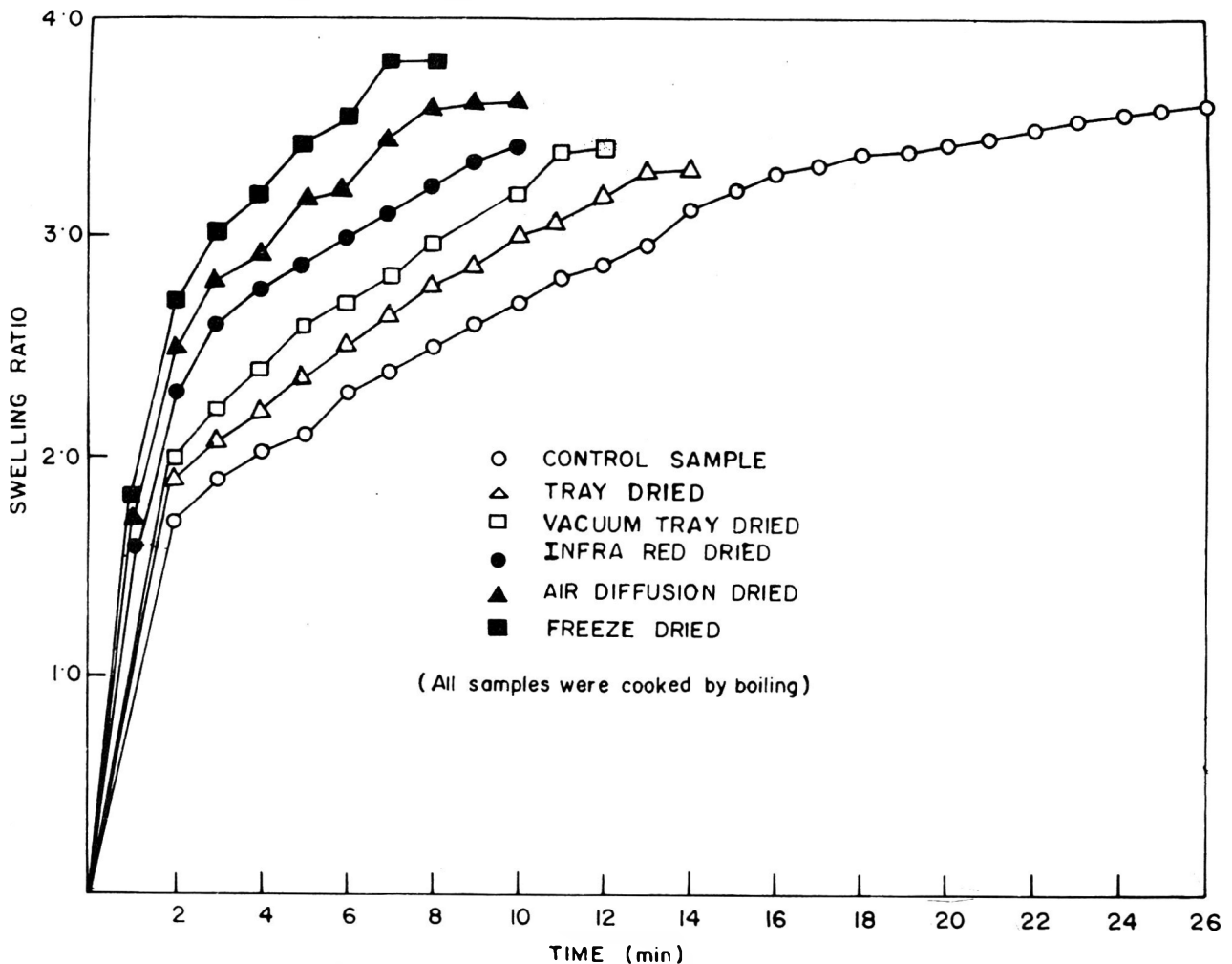


Fig 1 Swelling ratio of control and experimental samples

losses of vitamins were found to be highest in products dried in a tray dryer at atmospheric pressure.

Fig 1 shows the rate of water absorption in controls and samples dried in different dryers but cooked in boiling water only. It is noteworthy that the rate was progressively faster in the first two minutes of cooking of products obtained from different dryers in the following order; control, tray dried, vacuum tray dried, infra red dried, air diffusion dried and freeze dried samples. But the rate of water absorption was found to be relatively same in the subsequent periods as indicated by slopes of the curves. Similar studies made on products cooked partially or fully with steam prior to drying indicated similar characteristics in relation to the rate of water absorption.

When kept in sealed polythene bags (300 gauge) the dehydrated products showed no growth of fungus; there was also no deterioration in products quality when stored for 3 months in open shelves under normal conditions (temperature 20°–35°C and RH 55–95 per cent).

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Functional Properties of Raw and Cooked Moth Bean (*Phaseolus aconitifolius* Jacq) Flours

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Moth bean was cooked in an autoclave for 10, 15, 20, 25 and 30 min at 120°C and 0.705 kg/cm² pressure, (beans to water ratio was 1:4 wt/vol; beans were used as such), dried, ground and functional properties of flours were determined. Water and oil absorption capacity of flour were 2.4 g/g and 1.7 g/g, respectively. Cooking of bean for 30 min increased water and oil absorption capacity of flour by 12.5 and 52.9 per cent respectively. Gelation was found more or less constant. Nitrogen solubility vs pH profile showed only one minimum, at pH 4.5. Cooking lowered the nitrogen solubility at pH 2 to 12. The foaming capacity and emulsifying activity of bean flours were 27.0 and 20.2 per cent, respectively. Cooking for 30 min decreased the foaming capacity and emulsifying activity by about 48.1 and 31.0 per cent, respectively.

In recent years, there has been increasing interest in the functional potential of plant proteins. Legumes have been the focal point of this interest since they contain 18 to 25 per cent proteins. Moth bean (*Phaseolus aconitifolius* Jacq) has been identified as one of the potential food sources for the tropics¹. This bean is extensively grown in arid areas of India and some tropical countries and contains fairly a good amount of proteins, vitamins and minerals¹. Seeds of moth bean are often sprouted and cooked before they are consumed. Reports on a few functional properties of raw moth bean flours have been presented². However, information on functional properties of cooked moth bean flours is scanty³. The present investigation was, therefore, undertaken with this objective in mind.

Materials and Methods

Mature dry moth bean seeds (*Phaseolus aconitifolius* Jacq) grown and harvested in 1984 were purchased from the local market and cleaned.

Cooking: One hundred gram seeds were placed in 11 beakers and 400 ml of water were added, covered by aluminium foil and autoclaved for (10, 15, 20, 25 and 30 min). The temperature (120°C) and steam pressure (0.705 kg/cm²) were kept constant during autoclaving. The cooked beans were dried in a cabinet vacuum tray drier at an air velocity of 14 m/min and a temperature not more than 50°C to a final moisture content of 11 to 14 per cent, ground in a grinder, passed through a 0.25 mm sieve and stored at 4°C till analysis.

Proximate composition: Moisture, crude protein (N×6.25), crude fat and ash contents were determined using AOAC methods⁴. Total carbohydrates were

calculated by difference.

Functional properties: Water and oil (refined groundnut oil) absorption capacities were determined by the method of Beuchat⁵.

Least-gelation concentrations of moth bean flours were determined by the method of Coffmann and Garcia⁶ with slight modifications as described by Deshpande *et al*⁷.

Nitrogen solubility of moth bean flours was determined in the pH range of 2-12, as described by Narayana and Rao⁸.

Foaming capacity and foam stability of moth bean flours were studied according to the method of Coffmann and Garcia⁶. Foaming capacity as a function of pH (2-12) was also studied.

Emulsifying activity and emulsion stability were used as indices of emulsifying properties and were evaluated by the method of Yasumatsu *et al*⁹ with slight modifications as described by Deshpande *et al*⁷. The emulsion stability was evaluated by recentrifugation following heating at 80°C for 30 min in a water bath. Emulsification capacity as a function of pH (2-12) was also studied by the procedure of Beuchat *et al*¹⁰.

Unless otherwise mentioned, all the functional properties were studied at room temperature (28 ± 2°C) and values represent the average of three independent determinations.

Results and Discussion

Effect of cooking moth bean on proximate composition of flours: The data presented in Table 1 show that the moisture and total carbohydrates increased whereas a decrease in crude protein, crude

TABLE 1. EFFECT OF COOKING MOTH BEAN ON THE PROXIMATE COMPOSITION OF FLOUR^a

Constituents	Cooking time (min)					
	0	10	15	20	25	30
Moisture (%)	10.5	11.6	12.2	13.6	13.7	13.9
Crude protein (%) (N × 6.25)	23.5	23.4	22.1	21.0	20.6	19.4
Crude fat (%)	1.7	1.5	1.6	1.4	1.2	1.1
Ash (%)	3.7	3.6	3.6	3.4	3.2	3.1
Carbohydrate (%) (by diff)	71.1	71.5	72.7	74.8	75.0	76.4

^a On dry wt basis

TABLE 2. EFFECT OF COOKING MOTH BEAN ON THE WATER ABSORPTION OIL ABSORPTION AND GELATION PROPERTIES OF FLOURS

Cooking time (min)	Water absorption capacity (g/g)		Oil absorption capacity (g/g)		Least gelation concn (% w/v)
	Flour	Protein ^a	Flour	Protein ^a	
0	2.4	10.2	1.7	7.2	9.0
10	2.4	10.5	1.9	8.1	9.0
15	2.6	11.7	2.0	9.0	8.9
20	2.5	11.9	2.3	10.9	8.9
25	2.7	13.1	2.5	12.1	8.9
30	2.7	13.8	2.6	13.4	8.9
SE	±0.007		±0.026		±0.010
LSD					
(P=0.05)	0.023		0.081		NS

^a Expressed on crude protein basis

fat and ash content was noted in moth bean flour during cooking. The higher moisture content of the cooked and dried beans may be due to more moisture initially present in cooked beans. The slight lower values of crude protein, crude fat and ash in cooked samples may be due to the higher moisture values. The findings of the present investigation agreed fairly, with the reports on several mature dry legumes¹¹.

Effect of cooking moth bean on water and oil absorption capacity and gelation of flours: It was observed (Table 2) that the water and oil absorption capacity of flour was increased from 2.4 to 2.7 and from 1.7 to 2.6 g/g, respectively, during 30 min cooking. The corresponding increase in water and oil absorption capacity when expressed on crude protein basis was from 10.2 to 13.8 and from 7.2 to 13.4 g/g, respectively. It is possible that during cooking major storage proteins of moth bean, globulins, are dissociated into subunits and these subunits have more water binding sites than the native or oligomeric proteins. The carbohydrates may also play a role in water absorption. During cooking, gelatinization of the carbohydrates and swelling of the crude fibre may occur which could also lead to increased water absorption. The proteins denature during cooking and

the nonpolar residues from the interior of the protein molecule can unmask the fat which ultimately leads to increase in oil absorption. Similar increase in water and oil absorption capacity was reported on blanched mung bean flour¹² and on autoclaved winged bean flour⁸.

The least gelation concentration of raw and cooked moth bean flours was observed more or less constant (Table 2). del Rosario and Flores¹² reported 10 per cent gelation of raw and heat processed mung bean flours and did not find any difference. Coffmann and Garcia⁶ reported that gelation of mung bean protein isolates depends on protein concentration and that the gel formed by a 10 per cent concentration heated at 80°C for 10 min exhibited a more exceptional gelling ability than soybean proteinate. Results of present investigation showed that even the flours which had a little lower protein content formed stable gels at these conditions.

Nitrogen solubility: Nitrogen solubility vs pH profile of moth bean flours is depicted in Fig 1. Uncooked moth bean flour had minimum nitrogen solubility of 18 per cent around pH 4.5. On either side of this pH, it increased. Legume proteins in beans, whose major components are globulins, are known to be soluble in dilute salt solutions except at their isoelectric points. Protein solubility, however, is affected by heat treatment which results in protein denaturation. Therefore, nitrogen solubility decreased in cooked moth bean flours at all pH studied. The decrease was more conspicuous in 30 min cooked than the rest of the samples. In the present study, cooking denatured the proteins of moth bean flour and reduced their solubility in water at different pHs. Reduction in nitrogen solubility due to heat processing

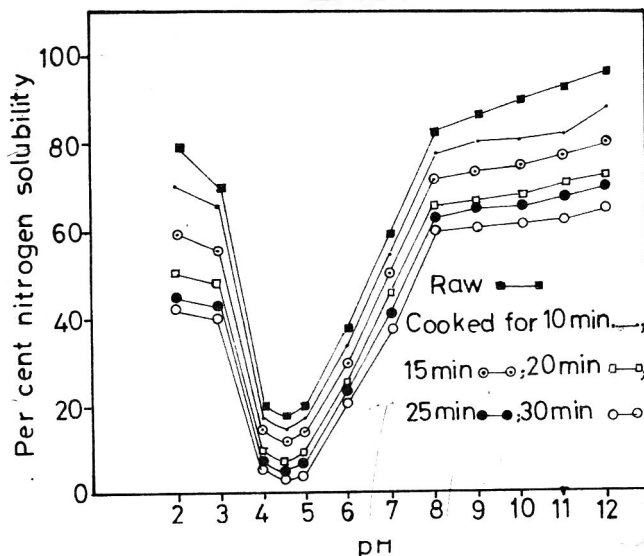


Fig. 1. Nitrogen solubility vs pH profile of raw and cooked moth bean flours.

TABLE 3. EFFECT OF COOKING MOTH BEAN ON THE FOAMING AND EMULSION PROPERTIES OF FLOURS

Cooking time (min)	Foaming properties			Emulsion properties			
	Vol increase (%)	Sp vol (ml/g)	Vol decrease over 120 min (%)	Emulsifying activity (%)		Emulsion stability ^a (%)	
				Flour	Protein ^b	Flour	Protein ^b
0	27	1.30	31.4	20.2	85.9	12.0	51.0
10	25	1.20	41.9	18.4	78.6	11.5	49.1
15	21	1.10	46.4	17.1	77.3	11.0	49.7
20	18	0.95	53.8	16.0	76.1	10.5	50.0
25	17	0.88	54.1	15.3	74.2	9.5	46.2
30	14	0.76	55.0	14.0	71.9	9.0	46.2
SE	±0.60	±0.009	±0.83	±0.033		±0.034	
LSD (P=0.05)	1.82	0.028	2.57	0.101		0.106	

a Per cent of the emulsifying activity after heating at 80°C for 30 min.

b Expressed on crude protein basis.

has also been reported in the case of winged bean⁸, soybean⁹, mung bean¹² and peanut¹³ flours.

Foaming properties: Cooking decreased foaming properties by about 50 per cent (Table 3). The per cent volume increase was decreased from 27 to 14 in 30 min cooked sample. Decrease in whippability and foaming property is correlated to the amount of native protein present. During cooking, proteins are denatured and thus foam capacity decreases. Cooking also decreased significantly the specific volume of foams from 1.30 to 0.76 ml/g during 30 min cooking. Same could be said of foam stability over 120 min. The decrease in foam volume over 120 min in 30 min cooked sample was 55 per cent as against 31.4 per cent in raw samples. It has been suggested that foam stability is also related to protein denaturation. Native proteins give higher foam stability than the denatured proteins. Similar observations on foaming properties have been reported on soybean⁹, mung bean¹² and winged bean proteins⁸.

The foam capacity vs pH profile of 2 per cent aqueous dispersion of raw and cooked moth bean flours (Fig. 2) closely resembled in shape its nitrogen solubility vs pH profile (Fig. 1) suggesting that foaming property of cooked samples was also dependent on the solubilized proteins. Minimum foam capacity of raw moth bean flour was observed to be 31.0 per cent at pH 4.5. Heat processing considerably lowered the foam capacity of moth bean flours at all pH studied. Yasumatru *et al.*⁹ have also reported diminished nitrogen solubility and foam capacity of soy proteins due to denaturation of proteins during heating. The increased foam capacity of cooked samples in acidic and alkaline pH may be due to increased solubility of proteins in these values.

Emulsion properties: During cooking, emulsifying activity decreased significantly from 20.2 to 14 per

cent for flour and 85.9 to 71.9 per cent for protein. The emulsion stability also dropped from 12.0 to 9.0 and from 51.0 to 46.2 per cent for flour and protein, respectively. The decrease in emulsion properties during cooking may be due to decreased solubility of proteins. McWatters and Holmes¹³ also reported similar results on soybean and peanut flour proteins.

The effect of pH on the emulsification capacity of moth bean flours is depicted in Fig 3. The emulsification capacity of all the cooked samples was decreased at all the pH studied. At pH of minimum solubility of protein (pH 4.5) emulsification capacity

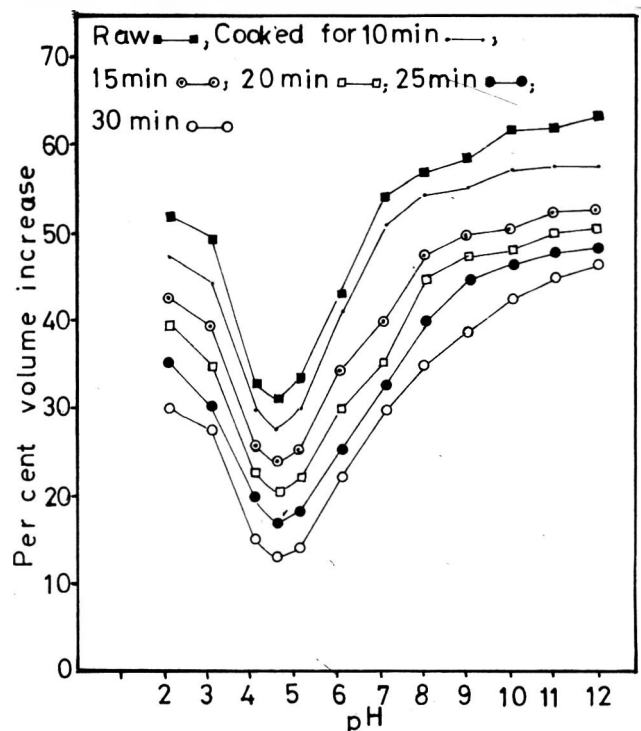
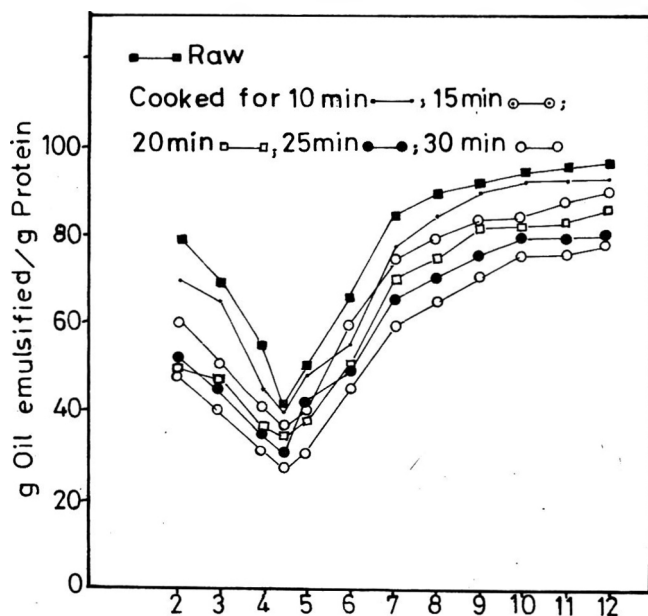


Fig. 2. Foaming capacity vs pH profile of 2 percent: W/V aqueous dispersions of raw and cooked moth bean flours.



g. 3. Emulsification capacity Vs pH profile of raw and cooked moth bean flours.

of 30 min cooked flour was only 26 g/g protein compared to 42 g/g protein for the raw flour which may be due to less protein available for unit oil to be emulsified. With either decrease or increase in pH, the emulsification capacity of all the samples increased suggesting that emulsification capacity of cooked samples was also dependent on solubilized proteins. The results of the present investigation agreed fairly with the results on winged bean⁵.

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Investigations on Phytate-Protein-Minerals Complexes in Whey Fractions of Moth Bean (*Phaseolus aconitifolius* Jacq) Flour

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Phytate, protein and minerals were determined from the whey fractions prepared from moth bean flour. The phytate-protein-minerals complexes were studied at pH 2.80, 6.40 and 8.40. Moth bean flour contained 23.5% protein and 0.74% phytate. About 87.7% of total phytate was found in water soluble form. Phytate phosphorus represented about 61.7% of total phosphorus. Recovery of phytate in whey fraction I (pH 2.80), fraction II (pH 6.40) and fraction III (pH 8.40) was 12.3, 0.8 and 17.6%, respectively after 2 days dialysis. At pH 2.80, phytate protein complexes occurred as a result of strong electrostatic interaction and at pH 8.40, complexation between phytate and proteins was mediated by divalent cations such as calcium, magnesium, iron and zinc. The concentrations of divalent cations were found higher in whey fraction III than I and II.

Phytate is widely present in foodgrains and processed food products¹. In most legume seeds, phytate phosphorus accounts for about 80 per cent of the total phosphorus² and is primarily present as a complex salt of minerals or complexed with proteins³. Phytate decreases the bioavailability of multivalent cations such as Ca^{++} , Mg^{++} , Zn^{++} and $\text{Fe}^{++}/\text{Fe}^{+++}$ and of proteins⁴ by forming phytate-minerals-protein complexes. Phytate-protein complexes are formed below the isoelectric point of the proteins⁴ and such complexes are insoluble and unavailable to human beings under normal physiological conditions. Changes in phytate phosphorus and minerals during germination and cooking of moth bean have been reported^{5,6}. The whey fraction contains a significant amount of water-extractable proteins, minerals and phytate. The high amount of phytate forms a complex with proteins and minerals and limits their utilization. The data on such complexes of phytate-protein-minerals which limit the utilization of proteins and minerals of moth bean are scanty. The present investigation was, therefore, undertaken with this objective in mind.

Materials and Methods

Seeds of moth bean were purchased from the local market, ground to pass through 60 mesh sieve and stored at 4°C until use.

Extractions and determination of phytate: Five extractants viz. distilled water (pH 6.40), tris buffer (0.05 M, pH 8.40), 3 per cent TCA (pH 1.40), 2 per cent HClO_4 (pH 0.80) and 2 per cent HCl (pH 0.80)

were used for extraction of phytate from moth bean flour. To determine the phytate content, a combination of three methods was used. Extraction and precipitation of phytate were performed according to the method of Wheeler and Ferrel⁷. The precipitate of ferric phytate was converted to ferric hydroxide and dissolved in 0.5 N HCl and made up to 100 ml with 0.1 N HCl as per the procedure of Makower⁸. The ferric iron (Fe^{+++}) content was determined by the AOAC⁹ method using O-phenanthroline reagent. Phytic acid (phytate) content was calculated on the assumption that it contained 28.2 per cent phosphorus.

Determination of protein: Nitrogen content was determined by the microkjeldahl method of AOAC⁹ and multiplied by 6.25 to obtain protein content.

Determination of minerals: Five hundred mg samples were acid digested with 12.5 ml nitric acid, 8.3 ml perchloric acid and 4.2 ml sulphuric acid mixture and diluted to a known volume (50 ml). From this hydrolysate, calcium, magnesium, iron and zinc were determined by atomic absorption spectrophotometry.

Extraction and preparation of whey fractions: Extraction and preparation of whey fractions were performed according to the method of Reddy and Salunkhe⁴. One hundred g of moth bean flour were extracted for 4 hr at room temperature with distilled water (1:10 flour to water, w/v). The slurry was centrifuged at $5,000 \times G$ for 40 min and the supernatant was collected. Residue was reextracted with distilled water for 2 hr at room temperature and centrifuged at $5,000 \times G$ for 40 min. The supernatants were combined and referred to as "water extract".

Water insoluble residue was washed twice with distilled water and lyophilized. The pH (6.40) of the water extract was adjusted to the pH of maximum precipitation (pH 3.50) with dilute HCl (0.1-0.5 N) and centrifuged at $5,000 \times G$ for 20 min. Insoluble residue at pH 3.50 was collected and freeze-dried; the supernatant remaining at pH 3.50 is referred to as whey fraction and was used for interaction studies between phytate, protein and minerals by dialyzing against three different pH solutions.

Four such whey fractions were prepared as mentioned above using 100 g flour each time. One sample was freeze-dried without dialysis and the other three were dialyzed for 48 hr with two changes against 20 volumes of 0.002 M HCl (pH 2.80), distilled water (pH 6.40) and 0.005 M tris buffer (pH 8.40), respectively. The dialyzed samples were centrifuged at $5,000 \times G$ for 20 min and the supernatants were freeze-dried and are referred to as fractions I, II and III.

Results and Discussion

Extraction of phytate from moth bean flour:

Extraction with 3 per cent TCA was more complete and yielded higher phytate than other extractants (Table 1). Moth bean flour had a phytate phosphorus content of 2.1 mg/g on dry weight basis (equivalent to 0.74 per cent phytic acid) and accounted for 61.7 per cent of the total phosphorus. Compared to complete solubilization of phytate phosphorus (2.1 mg/g) with 3 per cent TCA other extractants viz. 2 per cent HCl, tris buffer and 2 per cent HClO_4 solubilized 73.8, 76.1 and 81.9 per cent of the phytate, respectively. Phytate solubility in distilled water was 92.8 per cent. Low extractability of phytate with 2 per cent HCl and tris buffer may be due to formation of insoluble complexes between phytate, protein and minerals as reported for bean¹⁰, wheat protein concentrates⁷ and black gram⁴.

The data on recovered solids, protein and phytate during extraction and preparation of whey (Table 2)

TABLE 1. EFFECT OF DIFFERENT EXTRACTANTS ON THE EXTRACTABILITY OF PHYTATE FROM MOTH BEAN FLOUR^a

Extractant	pH of extract	Phytate P (mg/g)	Calculated phytic acid (mg/g)
3 % TCA	1.40	2.10 ± 0.07	7.44
2 % HClO_4	0.80	1.72 ± 0.04	6.09
2 % HCl	0.80	1.55 ± 0.05	5.49
Distilled water	6.20	1.95 ± 0.06	6.91
Tris buffer (0.05 M, pH 8.40)	8.30	1.60 ± 0.03	5.67

a Each value is the mean ± SD of six determinations and expressed on dry weight basis. Moth bean flour had a total phosphorus content of 3.40 mg/g (dry weight basis).

TABLE 2. DISTRIBUTION OF RECOVERED SOLIDS, PHYTATE AND PROTEINS IN WATER EXTRACT AND RESIDUE OF MOTH BEAN FLOUR^a

Sample	Recovered solids (g)	Phytate (mg/g)	% protein (N × 6.25)
Moth bean flour	100	7.44	23.50
Water extract	17.04	38.33 (87.78)	22.13 (16.04)
Water insoluble residue	76.30	0.55 (8.71)	33.41 (71.85)
Precipitate (pH 3.50)	5.70	41.50 (31.79)	44.46 (10.78)
Whey, undialyzed (pH 3.50)	11.34	36.73 (55.98)	10.90 (5.26)
Whey, dialyzed (pH 3.50)	3.00	2.64 (1.06)	33.81 (4.31)

a Each value is the average of three determinations. Values in parenthesis are the percentages of the original component recovered.

revealed that water extract contained 87.7 and 16.0 per cent of the original phytate and proteins (albumins), respectively. Lolas and Markakis² observed 99.6 per cent phytate in water-soluble form in beans compared to 87.7 per cent in moth bean flour in the present study. The water-insoluble residue contained 71.8 per cent water-insoluble proteins viz. globulins, prolamins, and glutelins and 8.7 per cent phytate. The water extract was rich in phytate and water-soluble proteins. When the water extract was adjusted to pH 3.5 (point of maximum insolubility) 67.2 per cent of the albumins precipitated along with 36.2 per cent phytates. The moth bean flour whey (pH 3.50) still contained 32.8 and 63.8 per cent water extractable proteins and phytates, respectively and used for interaction studies between phytate, protein and minerals at different pH conditions.

Influence of pH on phytate-protein-minerals complexes: The data on binding of phytate-protein-minerals at different pH and in different media as measured by dialysis (Tables 3 and 4) showed that fraction III contained maximum amount of protein (1.15 per cent) followed by fraction I (0.62 per cent) and fraction II (0.28 per cent) as against 10.90 per cent protein in undialyzed whey. The same could be said to phytate retention, being more in fraction III than in fractions I and II. The amount of phytate retained during dialysis was a function of pH. Dialysis data suggest that the retention of phytate was greater at the extreme pHs studied (Table 3).

At acidic pH 2.80 (below the isoelectric point of moth bean albumins) fraction I retained 22.1 per cent phytate during 2 days dialysis. Crean and Haisman¹¹ reported that of the 12 replaceable protons in the phytic acid molecule, 6 are strongly dissociated with

TABLE 3. BINDING OF PHYTATE WITH PROTEINS IN WHEY FRACTIONS I, II AND III AS MEASURED BY DIALYSIS AT DIFFERENT pH AND MEDIA

Sample	pH	Medium	Phytate (mg %)	Protein (g %)	% Phytate	
					retained after 2 days dialysis	mg/g protein at 2 days
Whey, undialyzed (pH 3.50)	-	-	36.73	10.90	-	3.36
Whey dialyzed Fraction I	2.80	HCl (0.002 M)	92.13	0.62	22.11 (12.38)	148.6
Fraction II	6.40	Dist water	6.38	0.28	1.53 (0.58)	22.8
Fraction III	8.40	Tris buffer (0.005 M)	131.10	1.15	31.47 (17.62)	114.0

a Each value is the average of three determinations. Values in parenthesis are the percentages of the original component recovered.

pk value of about 1.80 and 2 are weakly dissociated and had a pk value of about 6.30. Hence, phytate would exist as a strongly negatively charged molecule at pH values of 2.80, 6.40 and 8.40. Moth bean albumins, on the other hand, exist as positively charged molecules at pH 2.80 and a phytate protein binding was possible as a result of strong electrostatic interaction between positively charged parts of protein (lysyl, histidyl, arginyl and amino terminal groups) and negatively charged phosphate groups of phytate. Interaction between phytate and protein at acidic pH may also depend upon the number of positively charged groups available in the proteins that are free to react with anionic phosphate groups of phytate. Hence, as a result of phytate-protein complexes formation at acidic pH, 22.1 per cent of the phytates remained in the dialyzed fraction I and the ratio between phytate and proteins was highest, i.e. 148.6.

At pH 6.40 which is near the isoelectric point of moth bean proteins, the complexes between phytate and proteins did not form as indicated by 1.53 per cent retention of phytate during 2 days dialysis against distilled water. A major part of the phytate (about 98.4 per cent) was removed during dialysis. The low amount of phytate retained did not form a complex with proteins. At this pH, the phytate molecule also exists in the anionic form since it has pk value of about 1.80. While the proteins exist in neutral form in fraction II, the phytate to protein ratio was 22.8 (Table 3). Also, this fraction had appreciable amounts of divalent cations such as calcium, magnesium and iron (Table 4). Similar observations were reported on

TABLE 4. MINERAL CONTENT OF MOTH BEAN FLOUR, WHEY UNDIALYZED AND WHEY DIALYZED FRACTIONS I, II and III (MG/100G)^a

	Ca	Mg	Fe	Zn
Moth bean flour	200	232	10.5	3.5
Whey undialyzed	58.2	67.3	1.5	0.2
Whey dialyzed				
Fraction I	2.0 (3.4)	13.2 (19.6)	0.3 (20.0)	ND ^b
Fraction II	4.1 (7.01)	14.8 (21.9)	0.2 (13.3)	ND
Fraction III	25.4 (43.6)	30.5 (45.3)	0.9 (60.0)	ND

a Each value is the average of three determinations.

b Not detectable.

Values in parenthesis are the percentages recovered from the original water extracted components.

black gram⁴.

At alkaline pH 8.40 (above the isoelectric point of moth bean albumins) fraction III retained 31.4 per cent phytate after 2 days dialysis against tris buffer which may be due to association of phytate with proteins and/or minerals. At pH 8.40, both the phytate and proteins exist in ionized form (negatively charged). O'Dell and de Boland¹² reported that a strong phytate protein interaction occurs at high pH. Such interactions are mediated by divalent cations such as calcium and other ions¹³ when a certain minimum concentration of such cations is available for maintaining this complex¹⁴. In fraction III, the ratio of phytate to protein was 114.0. Fraction III had high concentrations of divalent cations i.e. 43.6, 45.3 and 60.0 per cent calcium, magnesium and iron, respectively, compared to the other two fractions viz fractions I and II (Table 4). These divalent cations might have been involved in complexation between phytate and proteins and therefore fraction III retained high concentrations of divalent cations i.e. calcium, magnesium and iron. Zinc, however, was not detectable in whey fractions.

It is clear from the observations that moth bean contained a significant amount of water extractable proteins, phytate and minerals. The phytate can form complexes with protein and minerals at acidic and alkaline pH. However, the complexes were negligible at intermediate pH.

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Efficacy of Vegetable Oils as Protectants of Greengram Stored in Different Jute Bags

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The present investigation was undertaken to find out the efficacy of four oils, namely, coconut, gingelly, groundnut and safflower oils as prestorage treatments for greengram. Greengram samples were separately coated with the above oils at 0.3 and 0.6% concentration and were stored in two types of bags (tightly knit and plastic lined jute bag) for six months. Though, there was a gradual increase in insect count, kernel damage, weight loss and uric acid content as the period of storage increased, in general, greengram samples treated with oils showed better resistance to insect infestation than untreated samples. Among the four oils used, safflower oil at 0.6% level was found to be effective with insignificant level of infestation. Among the bags, plastic lined jute bag was found to give better protection than the tightly knit jute bag for storage.

Producers of different pulses store 90 per cent of their produce at their households in rural areas of Andhra Pradesh¹. Seventy five per cent of them use bags; so it becomes crucial to advocate better methods of storage to reduce losses, specially at home level.

Insect infestation can be minimised or completely prevented by using various organophosphorus insecticides². But improvement in storage methods will be better accepted if existing storage practices are advocated with some modifications. In rural areas, simple traditional methods of control are used by farmers to protect their produce.

In recent years, the protective properties of certain edible oils against infestation of pulse beetle were evaluated³⁻⁵. The coating of stored pulses with a thin film of edible oil is a traditional method in some villages of India for protecting them against infestation by storage insects. Generally, the oil used for cooking in the area serves for treating the grains irrespective of the extent of protection it provides. Hence, this investigation was undertaken to find out the effectiveness of different types of vegetable oils as protectants for greengram stored in different types of bags against the attack of the pulse beetle which is a serious pest of stored pulses.

Materials and Methods

Selection of sample and treatments: Greengram (*Phaseolus aureus* Roxb), variety 'P-16', and two different tightly knit jute bags (one of them lined with plastic sheet) were selected for the study. The yarn count of the jute bag was 12/16 per square inch. Four oils, namely, coconut oil, gingelly (*Sesamum indicum* Linn) oil, groundnut oil and safflower oil were

selected for application to greengram as prestorage treatment.

Storage of sample: Thirty kg of freshly harvested greengram was collected from LAM research station, APAU, Guntur. The sample was subjected to prestorage treatments in the following manner. Greengram samples were coated separately with the above oils at 0.3 and 0.6 per cent level and were stored in two types of bags for six months. Untreated samples served as controls. All the samples were stored in triplicate. The bags were left on dunnage for 6 months.

Analysis: Moisture was estimated as per the standard procedure of A.O.A.C.⁶ Physical analysis (insect count and kernel damage) was done by the method of Pillai *et al*⁷. Weight loss was estimated according to the standard measurement technique given by Adams and Schuler⁸. Uric acid was estimated as per the procedure of Venkat Rao *et al*⁹. Aflatoxin was determined by the Tropical Products Institute standard procedure¹⁰.

Statistical analysis: The data obtained for all the parameters studied were subjected to analysis of variance as per Snedecor and Cochran¹¹.

Results and Discussion

Insect count of stored greengram: The fresh samples of greengram were completely devoid of visible insect infestation (Table 1). An increase in insect count was observed as the storage period progressed and this observation is similar to the reported values in literature¹²⁻¹⁶.

Lower level of insect infestation was observed in greengram stored in plastic lined jute bag when

TABLE 1. INSECT COUNT AND KERNEL DAMAGE OF STORED GREENGRAM TREATED WITH DIFFERENT OILS

Type of oil	Oil (%)	Live/dead insects (/100 g) in jute bags at indicated storage period (months)				Kernel damage (%) in jute bags at indicated storage period (months)			
		Tightly-knit		Plastic-lined		Tightly-knit		Plastic-lined	
		3	6	3	6	3	6	3	6
Untreated	—	112	200	30	41	27	38	12	26
Coconut	0.3	100	140	20	30	20	36	8	22
Coconut	0.6	85	120	19	28	17	30	6	16
Gingelly	0.3	46	99	14	22	14	27	1	8
Gingelly	0.6	36	70	10	20	7	20	1	7
Groundnut	0.3	97	152	18	26	17	38	8	20
Groundnut	0.6	70	100	16	25	14	38	4	14
Safflower	0.3	41	57	—	2	4	16	—	1
Safflower	0.6	26	46	—	2	2	14	—	1

Note: Initial sample: No insects and no kernel damage

compared to the insect count observed in samples stored in tightly knit jute bags. This may be due to the fact that plastic sheet might be acting as a barrier to the insect¹⁷. Murthy *et al*¹⁸ discussed the efficacy of plastic lined jute bag for storage of milled cereals. In the present study, the use of plastic lining did not completely prevent insect infestation in stored greengram.

Irrespective of the oil used, the insect count in the treated samples was found to be comparatively less than the untreated samples indicating that the oils inhibited the fecundity of pulse beetle.

Among the four oils, application of safflower oil at 0.6 per cent level was found to be the best prestorage treatment with lower level of insect infestation followed by gingelly oil, groundnut oil and lastly coconut oil. Some unknown factors present in safflower oil (and not present in other oils) might be more detrimental to growth and development of pulse beetle. Further research in this direction is needed.

The greengram samples treated with safflower oil

stored in plastic lined jute bag were fit for human consumption as they contained less than 10 insects/100 g which is the limit to the presence of insects according to PFA Act, 1954¹⁹.

Per cent kernel damage of stored greengram: Increasing trend in the percentage kernel damage in untreated samples (Table 1) on storage and with insect infestation was similar to the observations cited in literature on storage of pulses¹²⁻¹⁶.

The plastic lined jute bag was superior to the tightly knit jute bag. Maximum damage was recorded in untreated samples stored in tightly knit jute bags at six months.

In correspondence with the lower level of insect infestation in the stored greengram, among the four oils, application with safflower oil proved to be the best treatment with minimum kernel damage. Between two levels of oil treatment, safflower oil at 0.6 per cent level conferred maximum protection with minimum kernel damage and insect infestation.

Weight loss of stored greengram: The weight loss in

TABLE 2. PER CENT WEIGHT LOSS AND TRUE URIC ACID CONTENT OF STORED GREENGRAM TREATED WITH DIFFERENT OILS

Type of oil	Oil (%)	Weight loss (%) in jute bags at indicated storage period (months)				True uric acid (mg/100g) in jute bags at indicated storage period (months)			
		Tightly-knit		Plastic-lined		Tightly-knit		Plastic-lined	
		3	6	3	6	3	6	3	6
Untreated	—	14.9	25.6	8.7	13.0	188	335	50.3	68.7
Coconut	0.3	10.8	17.6	6.2	10.8	167	233	34.0	50.2
Coconut	0.6	8.8	14.0	2.5	7.3	142	201	32.0	47.0
Gingelly	0.3	7.0	13.0	0.6	4.3	79	162	23.5	38.0
Gingelly	0.6	3.5	9.9	0.3	3.1	59	117	16.8	33.2
Groundnut	0.3	8.6	18.9	4.4	7.7	163	221	30.0	43.0
Groundnut	0.6	6.9	16.5	2.3	6.9	117	168	26.8	41.9
Safflower	0.3	3.9	8.3	0.4	0.5	68	95	1.4	3.4
Safflower	0.6	1.3	7.8	0.2	0.4	43	60	1.2	2.3

Note: Initial uric acid: 0.9

stored greengram increased with the increase in storage period (Table 2).

Untreated greengram samples stored for six months in tightly knit jute bags showed maximum weight losses (25.6 per cent). Plastic lined jute bags offered better protection.

True uric acid content of stored greengram: The unhygienic quality of the grain is determined by the amount of uric acid present. It can be seen from Table 2, that the initial samples also contained negligible amounts of uric acid. This may be due to hidden infestation to which freshly harvested grain is easily prone. With the increase in storage period, the true uric acid content of stored greengram increased following the pattern of insect count and kernel damage. The highest uric acid content was recorded in untreated greengram stored in tightly knit jute bags at six months of storage (335 mg/100g).

The oil treated samples contained considerably lower true uric acid content, as the oils may have inhibited insect multiplication. The true uric acid content of samples treated with different oils differed, safflower oil being superior to other oils. Between the levels, of application of oils, safflower oil at 0.6 per cent showed maximum protection with minimum amount of true uric acid.

Between the two types of bags, the plastic lined jute bags, the plastic lined jute bag was found to be better as the true uric acid content of the stored greengram was less. This was in accordance with the lower level of insect infestation and per cent kernel damage observed in the samples stored in this bag.

It is observed from Table 2 that all the stored greengram samples except the safflower oil treated and stored in plastic lined jute bags contain true uric acid more than the standards (10 mg/100g sample) prescribed by Food Adulteration Act 1954 and thereby become unfit for human consumption.

Aflatoxin content of stored greengram: When the greengram samples were screened for aflatoxin, it was seen that none of the freshly harvested and samples stored at three and six months of storage was positive for aflatoxin contamination.

The results in this study indicate that safflower oil can be used for protecting pulses against pulse beetle even upto six months using a plastic lined jute bag.

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Study of Physical Characteristics, Sensory Evaluation and the Effect of Sprouting, Cooking and Dehulling on the Antinutritional Factors of Rice Bean (*Vigna umbellata*)

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The study of physical characteristics showed that rice bean having more length and weight had more edible portion as compared to mung bean. Sensory evaluation of cooked rice bean with and without sprouting was rated at par with cooked mung bean. Pressure cooking of rice bean at 15 lb for 20 min was more effective and time saving than open pan cooking. The effect of sprouting, cooking and dehulling on antinutritional factors revealed that on sprouting, the phytic phosphorus values for rice bean as well as mung bean decreased by 11.3 and 9.8%, respectively as compared to those of the whole ungerminated beans. The trypsin inhibitor activity of rice bean decreased by 44% on cooking and 30% on sprouting. The haemagglutinating activity in raw rice bean was 80 and steamed, sprouted and dehulled samples showed no residual haemagglutinating activity. Sprouting reduced the tannins in rice bean by 30.8% which were further reduced by 55% when sprouted rice bean was dehulled.

Pulses are important sources of protein in the diets of millions of people in Asia, Africa and South American countries. Protein content of pulses ranges from 17 to 25 percent, which is nearly twice that of cereals.

However, their contribution to the the nutrition of the consumer is limited, principally due to poor digestibility and antinutritional factors² which affect the utilization of dietary protein adversely. Several of these antinutritional factors can be eliminated or inactivated to a large extent by appropriate cooking schedules during food preparation. The treatments may include dehulling, presoaking, steaming and cooking³. An improvement in the biological value of soybean on germination has been reported⁴. Changes in the trypsin inhibitor activity and haemagglutinating activity have been found to occur during germination⁵.

Sprouting reduced the trypsin inhibitor activity by approximately 50 per cent in red kidney beans⁶. The haemagglutinin content in lentil seeds steadily decreased during germination and ultimately disappeared⁷. A good part of total phosphorus in legumes and cereals occurs as phytates which have been repeatedly shown to interfere in the absorption of iron, calcium, zinc and magnesium by forming complexes. Changes in the phytates during cooking, and germination are of significance in mineral nutrition of the consumer⁸.

The rice bean (*Vigna umbellata*) is a native of South and South East Asia and is cultivated by the tribals in various ethnic groups in the Eastern and North-

Eastern regions and to some extent in South India. It has a rich genetic diversity and high agricultural and nutritional potential⁹.

Since rice bean is a recent addition to pulses, its physical characteristics and sensory evaluation have been done. Further, the effect of sprouting, cooking and dehulling on the antinutritional factors present have also been studied.

Materials and Methods

A bulk lot of new variety of rice bean 'RBL-1', was obtained from the Department of Plant Breeding, Punjab Agricultural University, Ludhiana. Mung bean was obtained from the local market. The samples were freed of foreign material, if any, and kept in polythene bags and withdrawn as required. These samples were used for studying physical characteristics, sensory evaluation and the effect of sprouting, cooking and dehulling on the antinutritional factors after processing.

Physical characteristics: Grain colour, length, 1000-kernel weight and steeping characteristics of rice bean and mung bean were studied. The samples were soaked in tap water at room temperature (35°C) and increase in weight recorded. The colour of the samples was determined visually and the degree of cooking by a perceptive feeling of the cooked kernels.

Sensory evaluation: Rice bean and mung bean (100 g of each) were cooked in a pressure cooker at 15 lb for 20 and 15 min respectively using 225 ml and 200 ml of water. Salt (5g) was added to each sample during

cooking. Sprouted 'RBL-1' and mung bean (100g each) were steamed in a pressure cooker at 15 lb for 10 and 5 min, respectively, with 100 and 50 ml of water. Salt (5g) was added to each sample during cooking. The samples were served to a panel of 7 judges. Code numbers were given to different samples to avoid bias. The judges were asked to evaluate the cooked beans for colour, appearance, texture, flavour and overall acceptability by assigning scores as excellent (5), very good (4), good (3), fair (2) and poor (1). Degree of cooking was expressed as 'doneness' and it was scored as well cooked (3), overdone (2) and undercooked (1) respectively.

Preparation of experimental samples: Samples as prepared for sensory evaluation were taken for analysis. For dehulling, legumes were further steeped in excess of water for 2 hr and the hulls were gently rubbed off manually as customarily done in households. Proteins of the dehulled samples were dried at about 50°C and then analysed for antinutritional factors in triplicate.

Phytic phosphorus was determined according to the method of McCance and Widdowson¹⁰. The method of Kakade *et al*¹¹ was used for the determination of trypsin inhibitors. Trypsin inhibitor activity was expressed as per cent trypsin units inhibited. Haemagglutinating activity was determined by the method of Liener and Hill¹² while tannins were estimated according to AOAC¹³.

Results and Discussion

The grain characteristics of 'RBL-1' variety of rice bean as compared with mung bean are shown in Table 1. The hull content of rice bean was considerably less than that of mung bean. There was thus more of edible portion in rice bean than in mung bean. The endosperm of rice bean is of white hue as compared to the distinctly yellow colour of mung bean.

From the results in Fig.1, it is seen that mung bean tended to absorb more water at a faster rate than the rice bean. Water uptake (g/100 g dry matter) was found to be linearly related with time upto 7 hr of soaking unlike that of mung bean which was curvilinear. The pericarp of rice bean was found to be more tenaciously integrated to the cotyledons of mung bean.

TABLE 1. GRAIN CHARACTERISTICS OF RICE BEAN, VARIETY RBL-1, AND MUNG BEAN

Grain legume	1000 kernels wt (g)	Hull (%)	Length of 10 kernels (cm)	Colour
Rice bean	51.8	12.2	6.3	Greenish yellow
Mung bean	34.6	20.8	4.0	Deep green

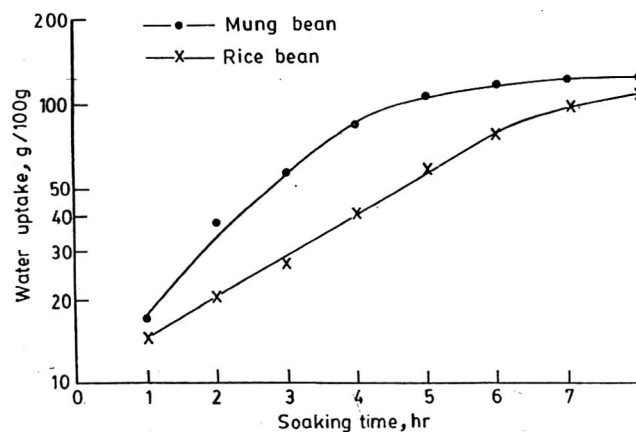


Fig. 1. Soaking characteristics of rice bean as compared to mung bean.

Regarding cooking characteristics, it took about half an hour longer to cook rice bean in boiling water than mung bean. The colour of rice bean changed altogether on cooking to chocolate while that of mung bean changed from a deep green to brownish. Pressure cooking of rice bean required 20 min as compared to 10 min for mung bean.

The differences in the average scores for colour, texture and flavour between rice bean and mung bean were nonsignificant, but were significant for appearance and overall acceptability (Table 2). However, significantly higher score was awarded to sprouted cooked rice bean than to cooked whole mung bean. Both the cooked grain legumes were graded 'good'. Since only a small panel was used, the results indicate the trend.

From the results in Table 3, it is seen that phytic phosphorus in mung bean exceeded that of rice bean by 37.5 per cent. Cooking did not affect phytic phosphorus content of either legumes. However, on sprouting the phytic phosphorus value for rice bean as well as mung bean decreased by 11.3 and 9.8 per cent respectively as compared to those of whole beans and there was a further reduction of 10.7 and 3.1 per cent on cooking them. The difference was attributed to the leaching effects during cooking. However, both the dehulled sprouted beans contained relatively higher values for phytic phosphorus which clearly indicated that there is more of phytic phosphorus in the cotyledons and less in the hulls (Table 3).

A marked reduction was observed in phytate phosphorus caused by germination of faba bean cultivars for 10 days¹⁴. Dehulling of dry beans significantly increased the phytic acid content of beans as reported earlier¹⁵.

The trypsin inhibitor activity compared to raw samples decreased by about 44 and 30 per cent on cooking rice bean and mung bean respectively (Table 4). However, complete destruction of trypsin inhibitor

TABLE 2. AVERAGE SENSORY SCORES FOR COOKED WHOLE AND SPROUTED RICE BEAN AND MUNG BEAN

Treatment	Appearance	Colour	Texture	Flavour	Overall acceptability
<i>Rice bean</i>					
Cooked	2.9	3.4	3.1	3.6	3.0
Sprouted, cooked	3.9	4.0	4.9	3.6	3.9
<i>Mung bean</i>					
Cooked	2.9	3.4	3.1	3.0	2.9
Sprouted, cooked	3.4	3.6	3.6	3.4	3.6
'F' ratio ^a	3.4	2.9	2.3	1.3	6.8
Least sig. diff ^b	1.5	0.9	1.5	1.3	1.0

Limits for average score: >4.6 – excellent; 2.6-4.5-very good; 3.6-3.5-good; 1.6-2.5-fair; <1.5 poor

^aTreatment variance/Error variance; for significance. P (0.05/0.01); 3.16/5.09 (3, 18 df)

^bLeast significant difference

TABLE 3. TOTAL PHYTTIC AND NON PHYTTIC PHOSPHORUS CONTENTS IN COOKED, SPROUTED AND DEHULLED RICE AND MUNG BEANS

Treatments	Total P (mg/100g)		Phytic P (mg/100g)	
	Rice bean	Mung bean	Rice bean	Mung bean
Raw	399.9	410.5	133.2	183.5
Cooked	400.8	412.0	130.6	181.6
Sprouted	400.0	411.5	119.5	165.5
Sprouted, cooked	401.1	412.0	106.7	160.4
Sprouted, dehulled	389.5	398.5	181.1	191.9
Sprouted, dehulled and cooked	389.9	399.7	180.8	190.8

TABLE 4. TRYPSIN INHIBITOR ACTIVITY (TIA) AND TANNINS IN COOKED, SPROUTED AND DEHULLED RICE BEAN AND MUNG BEAN

Treatment	TIA (%)		Tannins as tannic acid. (mg/100g)	
	Rice bean	Mung bean	Rice bean	Mung bean
Raw	13.94	15.26	993	612
Cooked	7.80	2.51	721	562
Sprouted	9.72	7.58	687	530
Sprouted, cooked	8.10	2.50	437	515
Sprouted, dehulled	17.70	17.70	306	368
Sprouted, dehulled and cooked	1.98	2.52	300	327

activity in *Phaseolus* species was possible by cooking for 5 min¹⁶. Since the results of the present study showed a slight decrease in trypsin inhibitor activity of rice bean during cooking, it is possible that residual activity may be due to inadequacy of cooking. For destroying trypsin inhibitor in soybean, more severe cooking is necessary¹⁷. The sprouted samples showed a decrease of about 30 and 50 per cent in trypsin inhibitor activity in rice bean and mung bean, respectively. Sprouted, dehulled samples showed higher trypsin inhibitor activity than sprouted ones (Table 4). Dehulling has been reported to increase trypsin inhibitor activity of dry beans by about 15 per cent¹⁵.

The rice bean variety contained considerably more tannins than mung bean (Table 4). In rice bean, the tannins were reduced by about 37.7 per cent as compared to a reduction of 8.2 per cent in mung bean. Sprouting further reduced the tannin content in both the beans. There was drastic reduction of tannins in sprouted dehulled samples of both the beans. Significant reduction in tannin content has been found with the removal of hulls from *Phaseolus vulgaris*¹⁵.

The haemagglutinating activities of rice bean and

mung bean were 80 and 320, respectively. However, steamed, sprouted and sprouted dehulled samples showed no residual haemagglutinating activity.

The investigations have shown that rice bean variety, 'RBL-1' is potentially a nutritive pulse. Further research for improving its cooking and dehulling properties and other methods to reduce the antinutritional factors in them is emphasized.

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Studies on the Occurrence, Partial Purification and Effect of Heating on Peroxidase in Some Vegetables

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Peroxidase activity was assayed in fifteen vegetables. The concentration of enzyme in vegetables was compared on wet and dry weight basis and found to vary widely among them. Studies on partial purification of the enzyme from some vegetables showed that acetone precipitation gave better recovery and higher fold purification than ammonium sulphate. Rate of loss of activity of the enzyme with heating was studied using the acetone precipitate and the activity-time curves obtained at different temperatures in the range of 60-100°C. 'D' and 'Z' values were established from the curves. Among the vegetables studied, the enzyme from lobia and cluster beans was found to have higher heat resistance than others.

Peroxidase (POD) (E.C.1.11.1.7, donor: hydrogen peroxide oxidoreductase) is known to be the most heat stable enzyme in vegetables. It has been known to withstand temperatures of boiling water and substantially above without permanent inactivation¹⁻⁴. It is among the most extensively investigated enzymes because of its wide distribution in nature especially in plants and its role in the formation of coloured products and off-flavour development in foods^{2,5-8}.

A wide variation in the level of peroxidase activity among several vegetables has been reported earlier⁹. Any undesirable changes in colour and flavour on storage are due to inadequate blanching prior to processing^{10,11}. Inactivation curves for the enzymes have been reported to exhibit two linear sections corresponding to a rapid inactivation phase and a highly delayed phase^{12,13}.

Studies reported so far in literature are confined to a limited number of vegetables such as horseradish, spinach, potato, tomato, etc. No attempt has been made so far to screen the different types of vegetables cultivated in India for peroxidase activity and concentration and also evaluate the enzyme from the different sources for its heat resistance. Such a study is important with a view to laying down the minimum heat inactivation times for the different vegetables used for further processing like freezing, dehydration, canning etc., to ensure quality during storage.

In the studies reported here, 15 locally available vegetables were screened for the enzyme activity and concentration and the enzyme from vegetables exhibiting high concentration was partially purified and its thermal resistance characteristics was studied in terms of 'D' and 'Z' values.

Materials and Methods

Raw Materials: Following fresh vegetable varieties available in the local market were used.

Cabbage (*Brassica oleracea* var. *Capitata* Linn)-white cabbage, cauliflower (*Brassica oleracea* var. *Botrytis* Linn)-Indian cauliflower, Knol Khol (*Brassica oleracea* var. *Gongylodes* Linn)-White Vienna, Brinjal purple-'Arka Sheel', Brinjal green-'Arka Shirish', Japanese radish-'Japanese White', Horseradish-'Chinese pink', French bean-string type, Lobia (*Vigna Catjang*)-'Pusa Dofosli', cluster bean-'Pusa Sadabahar', Onion-'Pusa Red', green peas-'Bangalore local', pumpkin-'Arka Chandan'.

Extraction of peroxidase: Four grams of the cut plant tissue were homogenised in 40 ml of 0.05 M cold citrate-phosphate buffer (pH 5.0) for 5 min in a high speed homogeniser (Virtis, USA). The extracts were filtered through cheese cloth, centrifuged at 0°C and supernatant was used for further partial purification and heat inactivation studies.

Assay of enzyme activity: Peroxidase activity was assayed according to Mihalyi *et al*⁹. The enzyme was assayed colorimetrically. The reaction mixture contained 1 ml enzyme extract, 5 ml citrate (0.025M) - phosphate (0.05M) buffer (pH 5.0) and 1.5 ml of 0.1 per cent H₂O₂. The reaction was started by adding 2 per cent O-phenylene diamine and the absorbance at 450 nm was followed for 3 min in a Spectronic 20 colorimeter. One unit of peroxidase activity was defined as of 1 absorbance unit increment per min at 450 nm. Protein concentration in the extract was measured by the method of Lowry *et al*¹⁴. using crystalline bovine serum albumin as standard.

Partial purification of crude enzyme extract: Filtrate from the crude vegetable extract was centrifuged at $15,000 \times G$ for 20 min at $0^{\circ}C$. The enzyme was precipitated by adding 1.6 volumes of acetone at $-20^{\circ}C$ and stirring the mixture for about 10 min at $-10^{\circ}C$ (freezing mixture). The precipitate was separated by centrifugation at $15,000 \times G$ for 10 min at $-10^{\circ}C$, dissolved in buffer, kept overnight at $0^{\circ}C$ and centrifuged at $20,000 \times G$ for 20 min to remove the inactive residue. The clear supernatant so obtained was used as partially purified enzyme. It was maintained at $0^{\circ}C$ until used.

In the preliminary studies, the crude enzyme extract was precipitated at $0^{\circ}C$ by saturation with calculated amounts of solid ammonium sulphate into various fractions, namely 0-50 per cent and 50-90 per cent. The precipitate from each fraction was collected, dissolved in a suitable volume of phosphate-citrate buffer (pH 5.0) and assayed for peroxidase activity and protein.

Study of heat resistance of the enzyme: About 0.5 to 1.0 ml of the partially purified enzyme extract after suitable dilution was placed in a pre-warmed test tube

of dimensions 13 mm O.D. and 20 cm long, kept immersed in a water bath at the required temperature. At each temperature, a known volume of the heated enzyme was withdrawn at different time intervals, immediately cooled by transferring to a test tube kept in an ice bath and the activity assayed as described above. 'D' values were obtained by plotting per cent relative activity against time for each temperature ranging from $60-100^{\circ}C$ on semi-logarithmic paper. 'Z' values were obtained from a plot of 'D' value vs temperature on semi-logarithmic paper. Purified horseradish peroxidase (RZ = 2.0) obtained from Sigma Chemical Company, USA, was used for comparison.

Results and Discussion

Enzyme concentration: Peroxidase concentration, moisture and pH of various vegetables are given in Table 1. The level of enzyme activity was found to vary considerably with the type of vegetables; cluster beans, lobia, horseradish, cabbage and knol-khol possessing higher concentrations. Moisture content varied from 72 to 96 per cent and pH from 4.5 to 6.6. Variation in peroxidase concentration among

TABLE 1. PEROXIDASE CONCENTRATION OF VEGETABLES

Vegetables	Moisture (%)	pH	Peroxidase concn* (units/ g sample)	
			Wet wt. basis	Dry wt. basis
Cluster beans (<i>Cyamopsis tetraganolaba</i>)	86.0	6.1	50.3 ± 9.0	359 ± 64.0
Horseradish (<i>Armoracia laphathifolia</i>)	90.0	5.1	40.0 ± 6.5	400 ± 65.0
Lobia (<i>Vigna unguiculata L.</i>)	80.0	5.6	30.4 ± 4.5	152 ± 20.4
Cabbage (<i>Brassica oleracea L.</i>)	93.5	5.7	30.0 ± 1.8	462 ± 30.0
Knol-khol (<i>Brassica oleracea L.</i>)	93.1	6.2	21.0 ± 1.8	304 ± 28.4
Cauliflower (<i>Brassica oleracea L.</i>)	89.4	6.2	15.5 ± 1.2	146 ± 12.2
Kovai tender (<i>Coccinia indica</i>)	93.4	4.8	12.0 ± 3.9	182 ± 45.2
Japanese Radish (<i>Raphanus Sativus L.</i>)	95.0	5.2	10.1 ± 2.2	202 ± 40.4
Brinjal (green) (<i>Solanum melongena L.</i>)	91.3	5.0	3.8 ± 1.0	44 ± 11.0
Brinjal (violet) (<i>Solanum melongena L.</i>)	92.3	5.3	3.3 ± 0.8	43.6 ± 10.4
Peas Green (<i>Pisum sativum L.</i>)	72.0	6.6	1.9 ± 0.5	6.7 ± 2.1
Beans French (<i>Phaseolus vulgaris L.</i>)	90.8	6.0	0.7 ± 0.2	8.4 ± 2.4
Onion (<i>Allium cepa L.</i>)	86.5	5.7	0.3 ± 0.1	2.3 ± 0.7
Pumpkin (<i>Cucurbita pepo</i>)	85.3	6.2	0.2 ± 0	1.5 ± 0
Ash gourd (<i>Benincasa hispida</i>)	96.2	4.5	0.15 ± 0	3.9 ± 0

*Figures (mean ± s.d.) are the mean of six replicates drawn from six different lots.

TABLE 2. FRACTIONATION OF CLUSTER BEAN PEROXIDASE

Mode of extraction/ purification	Volume (ml)	Total activity (units)	Total protein (mg)	Sp activity (units/mg protein)	Recovery (%)	Fold puri- fication
Citrate-phosphate buffer Ammonium sulphate	40	190.3	124	1.5	100.0	1.00
0-50%	10	4.2	18.6	0.23	2.21	0.15
50-90%	10	160	24.8	6.4	84.07	4.20
Acetone precipitate	20	177	20.0	8.9	93.30	5.79

TABLE 3. ACTIVITY, PROTEIN AND SPECIFIC ACTIVITY OF PARTIALLY PURIFIED* PEROXIDASE FROM SOME VEGETABLES

Vegetables	Activity (ur.its/ml)	Protein (mg/ml)	Sp activity** (units/mg protein)	Fold purification	Recovery (%)	
					Acetone ppt	Ammonium sulphate ppt (50-90%)
Lobia	5.8	0.3	17.6(2.1)	8.8	96.5	30.8
Cluster beans	13.3	1.0	13.3(1.1)	12.1	93.3	84.1
Cabbage	11.1	0.4	30.7(2.5)	12.3	83.4	25.1
Cauliflower	4.6	0.7	7.0(1.2)	5.8	80.0	29.6
Horseradish	14.4	0.3	54.3(4.7)	11.4	79.0	29.8
French beans	1.0	0.9	1.2(0.2)	5.9	78.6	26.0
Japanese radish	3.3	0.1	26.8(5.0)	5.4	73.5	29.0
Kovai tender	1.5	0.6	2.0(1.1)	1.8	63.8	29.4
Knol-khol	3.3	0.2	17.3(3.0)	5.7	55.6	14.3

* Using acetone precipitation

** Figures in parentheses are the specific activity of the corresponding crude buffer extract.

vegetables has been reported earlier also⁹.

As indicated in Table 2, 50-90 per cent ammonium sulphate fractionation gave the best recovery of the enzyme and the specific activity was 4 fold. Acetone precipitation was much better with 93 per cent recovery and six fold purification.

Table 3 gives the activity, protein content, specific activity and purification of acetone purified peroxidases from some vegetables. The data show that POD from cluster beans, horseradish and cabbage gave higher fold purification and kovai tender gave the least.

Heat inactivation: Results of studies conducted on the thermal stability and kinetics of heat inactivation of the partially purified enzyme extracts from some of the vegetables and also of pure horseradish peroxidase are given in Table 4. 'D' values were obtained by calculating the time required at a given temperature to reduce the activity to 10 per cent of the initial value. A plot of 'D' vs temperature provided the TDT curves or the 'Z' value. It was found that there were two linear

TABLE 4. 'D' AND 'Z' VALUES FOR PEROXIDASE IN SOME VEGETABLES

Vegetables	'Z' value (°C)	'D' value (min)				
		60°C	70°C	80°C	90°C	100°C
Lobia	30.0	—	12.9	6.0	4.2	1.4
Cluster beans	30.0	—	10.0	4.8	1.9	1.1
Horesradish	23.0	10.0	7.6	1.0	0.5	Nil
Cabbage	20.3	4.8	2.4	0.4	Nil	Nil
Kovai tender	20.0	5.0	3.0	1.0	Nil	Nil
Japanese radish	16.9	4.0	0.8	0.2	Nil	Nil
Knol-khol	15.7	2.2	0.75	Nil	Nil	Nil
French beans	13.6	2.2	1.3	0.7	0.4	0.23
Cauliflower	10.0	1.6	1.2	0.6	Nil	Nil
Horseradish peroxidase*	25.3	—	11.1	3.2	1.5	0.65

*Purified (sigma)

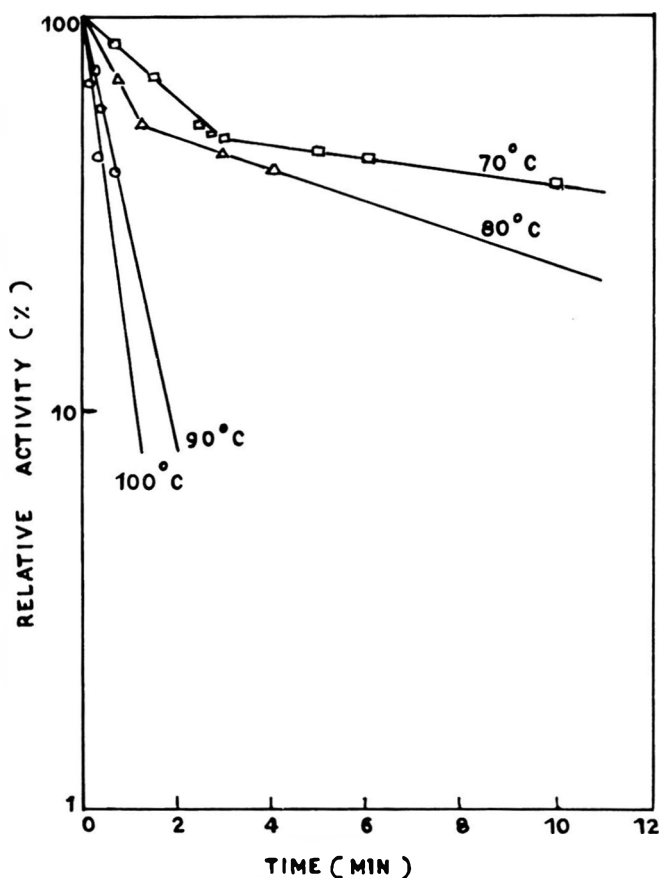


Fig. 1. Heat inactivation of peroxidase from cluster beans.

sections in the heat inactivation curve, one a rapid inactivation phase and the other a highly delayed phase. It has been reported that the break in the heat inactivation curve is due to two fractions of peroxidase which had different degrees of heat resistance, a heat sensitive peroxidase fraction and a heat resistance peroxidase fraction¹². Figures for

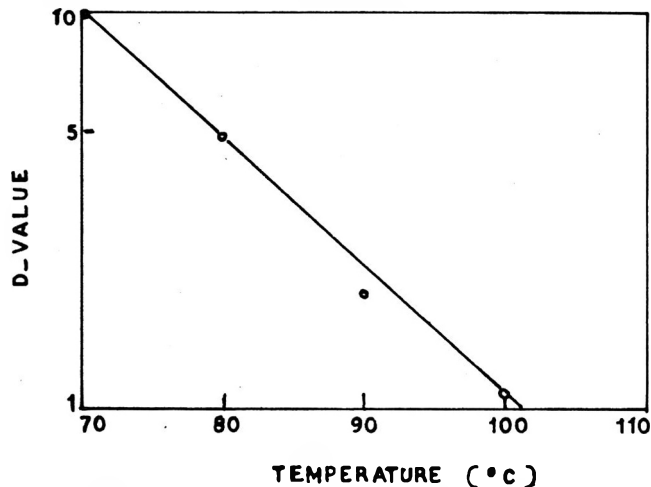


Fig. 2. Thermal destruction rate curve for inactivation of cluster bean peroxidase.

cluster beans are given (Fig. 1 and 2) to illustrate this point. Similar results have been reported earlier in the case of spinach peroxidase¹² and also in purified and crystallised horseradish peroxidase¹³. At 70 and 80°C the first part of the curve only has been used to evaluate 'D' values after extrapolation.

'Z' values reported in literature for horseradish peroxidase vary widely. The values obtained in this study were slightly lower (25.3°C) than the reported value (27.3°C)¹⁵. Again in our study, the partially purified horseradish peroxidase was found to give a lower 'Z' value (23.0°C) than the purified enzyme obtained from Sigma (25.3°C).

Thus, peroxidases from cluster beans and lobia are less heat sensitive than enzymes from other vegetables including horseradish as they possessed higher 'Z' values and cauliflower peroxidase was the most heat sensitive enzyme.

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Studies on Maturation of Apples: Effect of Seasonal Variation on Physico-chemical Parameters and Their Correlations

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Growth and maturation pattern of four cultivars of delicious apples grown in Himachal Pradesh were studied for seasonal variations during four consecutive years. All the physico-chemical maturity parameters employed showed clear changes during the maturation period and seasonal variations. Among them, the disappearance of starch with a starch pattern index of 4.0 and browning of seeds with a seed colour index of 6.0 together clearly define optimum harvest maturity. Making use of them, limits for optimum harvest maturity as affected by seasonal variations were determined for diameter, weight, total soluble solids, acidity and MT puncture values. All the maturity parameters considered showed highly significant linear correlation among one another. Apples harvested at optimum maturity showed good quality and storage life. The methodology will be useful as a quality control-measure of fresh produce and in the study of seasonal, varietal and regional differences in apple quality.

Several methods based on employing physical, physiological and chemical parameters like fruit ground colour, size, ease of separation, fruit pressure, climatic conditions (number of days elapsed from full bloom, heat units, etc.), and the changes in the chemical constituents like total soluble solids, acidity and starch have been reported¹⁻⁸ for judging maturity of apples.

In all these studies, only a few of the internal quality parameters and/or external factors (agro-climatic conditions) have been taken into consideration. No attempts have been made to study these parameters exhaustively and use them to define clearly the harvest maturity, the influence of harvest maturity on fruit quality and to establish correlations among them. In the present investigation, ten different maturity parameters of the fruit itself, viz. visual skin colour, diameter, weight, density, respiration, fruit pressure, seed colour, acidity, total soluble solids and disappearance of starch were studied on four important commercial varieties of apples for four successive seasons (1973-76). They were used to define optimum harvest maturity clearly fixing limits and correlations were worked out.

Materials and Methods

Apple samples: An apple orchard spread over 12 ha, located in the village Thanedhar, latitude 38°, longitude 76°, 2270 m above sea level in Simla Hills of the Himachal Pradesh was chosen. The slope of the hill is in the north to south direction at this location. Four cultivars of apples viz. 'Royal Delicious', 'Red

Delicious', 'Rich-a-Red' and Golden Delicious' were planted intermingled. For each variety, 12 healthy and uniform bearing trees medium size around 20 years of age with at least 6 m height were selected at random from a continuous area of about 1.25 ha of the orchard within an altitude variation of about 30 m from the bottom most tree to the top most tree. During the four years period, the selected trees were given similar mineral nutrition with the usual prophylactic treatment against diseases as practised in the orchards.

Uniform sized apples located in the periphery of the tree upto a height of about 2 m from the ground were tagged with paper tags, harvested between 100 and 150 days after full bloom and used for different maturity tests. Eight weekly harvests were made in each year. At each harvest 120 fruits, 10 fruits per tree × 12 trees out of 1000 to 1500 fruits borne per tree were picked. The fruits from each tree were collected individually in a sack, labelled, brought and analysed in the laboratory at Thanedhar on the same day.

Physical and chemical tests of maturity: Colour, diameter, weight, density, starch-iodine test and seed colour test assessments were done on all the 120 fruits. Before starch-iodine test and seed colour test, respiration of 12 fruits (1 fruit/tree) in 3 replicates of 4 fruits and fruit pressure on 60 fruits (5 fruits/tree) were taken.

Diameter, weight and density: The diameter by using vernier calipers, weight by double pan Avery balance and volume by water displacement were determined. Density was computed from weight and volume data.

Fruit pressure: The fruit pressure was measured using a Magness-Taylor (MT) puncture tester with a plunger of 5/16 of an inch diameter. On each fruit two observations as pressure just adequate to force the plunger of 5/16 of an inch diameter. On each fruit, two 7/16 of an inch, were taken and the mean pressure was expressed as lb/sq. in.

Total soluble solids (TSS): Juice expressed from the composite samples was analysed for the percentage TSS using a hand refractometer.

Acidity: The acidity was estimated on composite samples by standard method and expressed as per cent malic acid per 100 g fleshy part of the fruit.

Respiration: The respiration rate was determined according to the continuous current method of Loomis and Shull⁹.

Visual skin colour: During growth and maturation, the red coloured varieties which have an initial green colour picked up intermediate hues of red and green colour as the growth and maturation progressed and attained deep red finally. However, the extent of deep red colour formed on the surface of the fruits depends on its exposure to direct sunlight. These changes in the visual skin colour of apples were grouped into five stages as follows: Stage 1-100 per cent green (assigned point 1), Stage 2-50 per cent green and 50 per cent red (point 2), Stage 3-20 per cent green and 40 per cent greenish red and 40 per cent red (point 3), Stage 4-100 per cent red (point 4) and stage 5-100 per cent deep red (point 5). In 'Golden Delicious' apples, the fruits were initially light green in colour which turned into yellowish green, greenish yellow, pale yellow and finally into golden yellow. These were recorded and assigned points similarly for the five stages. A visual skin colour index (VSCI) as a weighted average using the percentage of fruits and points corresponding to different stages was worked out e.g., the percentages of number of fruits falling under the above five stages were 7, 5, 62, 15 and 11; the corresponding VSCI will be $(7 \times 1 + 5 \times 2 + 62 \times 3 + 15 \times 4 + 11 \times 5) / 100 = 3.18$.

Seed colour: The changes in the colour of the seed from white in the immature stage to brown in the mature stage which take place by gradual browning from the micropilar end and spreading to the whole seed with the advancement in maturation was recorded by observing the colour of the seed. Six stages of browning of seed viz. 10, 25, 50, 75, 90 and 100 per cent (points 1-6) were identified based on the percentage of brown coloured area of the seed. A seed colour index (SCI) was worked out similarly as weighted average between the percentage of seeds in each stage of browning and their corresponding points¹⁰.

Starch-iodine test: The rate of disappearance of

starch from the core region in the equatorially cut apple slices was tested as described by Krishnaprakash *et al*¹¹. A starch pattern index (SPI) was worked out using the percentage of fruits in each category of maturity and their corresponding points as a weighted average¹⁰.

Sensory evaluation: To establish relationship between eating quality and harvest maturity as measured by SPI, only 'Red Delicious' apples during the first two seasons were studied. A separate set of 360 fruits (30 fruits per tree \times 12 trees) under each of the three different stages of maturity assessed to be immature (stage 1), mature (stage 2) and overmature (stage 3) with an average value of SPI ≤ 3.0 , 3.5-4.5 and ≥ 5.0 , respectively were harvested. These were packed in 9 wooden crates (3 crates \times 3 stages) with cell pack and transported to Mysore by railway. On arrival of the fruits at Mysore after 10 days, they were cold stored at $32 \pm 1^\circ\text{F}$, 90 ± 5 per cent RH. Fruits were evaluated as on arrival, and after 3 and 5 months cold storage by 20 trained panelists on a descriptive quality profile procedure¹².

Statistical analysis: The sensory data were analysed by analysis of variance followed by Duncan's new multiple range test¹³. Each of the ten maturity parameters was plotted against the number of days of maturity in a graph. Multiple linear regression (MLR) between selected parameters and correlation coefficients among all the parameters were calculated.

Results and Discussion

The results of sensory evaluation of 'Red Delicious' apples are presented in Table 1. The mean scores for sensory attributes during the first season indicate that on arrival the quality of fruits belonging to maturity stages 2 (mature) and 3 (overmature) were comparable whereas the quality of stage 1 (immature) was slightly inferior being on the unripe side. At 3 months cold storage, the stage 2 fruits were significantly different and superior in all the quality attributes to the other two stages which were comparable in quality but almost reached the limit of cold storage life. This tendency became very clear in 5 months stored fruits where stage 2 fruits remained significantly superior, acceptable and still had cold storage life as compared to the fruits of the other two stages which were comparable, inferior and of unacceptable quality. The inferior quality of the fruits in stage 1 is mainly due to the lack of development of aroma and taste qualities and in stage 3 due to the texture breakdown accompanied by poor juiciness, their respective scores being significantly higher than the stage 2 fruits. Stage 2 fruits showed desirable qualities in all the sensory attributes, mean scores

TABLE 1. QUALITY AND STORAGE BEHAVIOUR OF RED DELICIOUS APPLES HARVESTED AT DIFFERENT STAGES OF MATURITY DURING THE FIRST AND SECOND SEASONS

Harvest stage	Starch pattern index	Storage period (months)	Texture	Juiciness	Aroma	Taste	Overall quality
<i>Season 1</i>							
1.	3.0	On arrival	3.5 ^b	3.2 ^a	2.9 ^a	2.8 ^a	2.9 ^a
2.	4±0.5	On arrival	3.1 ^a	3.2 ^a	3.3 ^b	3.2 ^b	3.2 ^b
3.	5.0	On arrival	3.5 ^b	3.2 ^a	2.9 ^b	3.2 ^b	2.8 ^a
1.	-	3	5.1 ^b	5.2 ^b	5.4 ^c	5.4 ^c	2.7 ^a
2.	-	3	4.7 ^a	4.8 ^a	4.8 ^a	4.8 ^a	3.3 ^b
3.	-	3	5.5 ^c	5.3 ^b	5.1 ^b	5.1 ^b	2.6 ^a
1.	-	5	5.5 ^b	5.6 ^b	5.9 ^c	5.8 ^c	1.9 ^a
2.	-	5	5.1 ^a	4.9 ^a	5.0 ^a	5.2 ^a	3.0 ^b
3.	-	5	5.8 ^c	5.9 ^c	5.4 ^b	5.5 ^b	2.1 ^a
<i>Season 2</i>							
1.	3.0	On arrival	3.6 ^a	3.5 ^a	3.2 ^a	3.2 ^a	3.1 ^a
2.	4±0.5	On arrival	3.7 ^a	3.4 ^a	3.6 ^b	3.7 ^b	3.5 ^b
3.	5.0	On arrival	3.8 ^a	3.4 ^a	3.4 ^{ab}	3.8 ^b	3.5 ^b
1.	-	3	5.3 ^b	5.4 ^b	5.7 ^c	5.6 ^c	2.5 ^a
2.	-	3	5.0 ^a	4.9 ^a	5.0 ^a	5.0 ^a	3.3 ^c
3.	-	3	5.3 ^b	5.3 ^b	5.4 ^b	5.3 ^b	2.8 ^b
1.	-	5	5.4 ^b	5.5 ^b	5.9 ^c	5.9 ^c	2.1 ^a
2.	-	5	5.1 ^a	5.1 ^a	5.2 ^a	5.3 ^a	2.9 ^b
3.	-	5	5.7 ^c	5.8 ^c	5.5 ^b	5.6 ^b	2.3 ^a

Sensory mean scores for individual quality attributes: 3.5 = Possibility of quality improvement, 3.5-4.5 = optimal quality 5.5 = Limit for cold storage life: Overall quality: 5 = Excellent, 4 = Very good, 3 = Good, 2 = Fair, 1 = Poor.

Mean scores carrying different superscripts in each column in each season under each storage period differ significantly ($P \leq 0.05$).

being within the range of good to fair till the end of the 5 months cold storage period. The data obtained in the next season confirmed these findings.

Thus, a significant relationship between maturity and quality was established. Immature fruits with an average value of SPI ≤ 3.0 , showed inferior quality and poor storage life, mature fruits with an SPI range of 3.5-4.5, superior quality and longer shelf life and over-mature fruits with an average value of SPI ≥ 5.0 , showed inferior quality and poor storage life. This indicates that fruit maturity at harvest plays an important role in influencing the quality and storage of apples. The results obtained in our preliminary studies reported earlier¹⁴ were also confirmed by these findings.

Each one of the ten maturity parameters against days of maturity is presented in Fig. 1,2,3,4 and 5 which show their pattern of changes during the maturation period and also their distinct seasonal variations, the individual graphs not overlapping. Clear and well defined pattern of changes are observed in most of the maturity parameters. Diameter, weight, TSS, starch pattern index, SCI and VSCI showed steadily increasing trend whereas acidity and MT puncture values showed a decreasing trend. These parameters showed significant linear correlation

among one another. However, density and respiration did not exhibit a definite pattern in relation to maturity.

Apple being a plantation crop, the fruits cannot attain uniform maturity at the same time. For an optimum harvest maturity, an ideal case is when the average SPI=4.00, a standard case is when the average SPI=3.75 – 4.25 and a limiting case is when the average SPI=3.5 – 4.5. A rational distribution of these is given in Table 2. With regard to SCI, a value of 5 will indicate slightly immature (SIM) stage and 6, mature (M) stage but values beyond 6 cannot be taken to represent overmature stage. Hence, it is not possible to work out a distribution with SCI.

Dhanaraj *et al*¹⁰, by making use of the above concept in their study on the effect of elevation on maturity and quality of apples, have statistically determined the actual number of days required to attain optimum harvest maturity. Later, Narasimham *et al*¹⁵, in their study on the effect of meteorological conditions on maturation of apples used MLR between SPI, SCI and days of maturity and determined optimum harvest periods as a practical range of days because apple being an agricultural commodity considering a particular day as optimum for harvest is not practical; it can only be determined

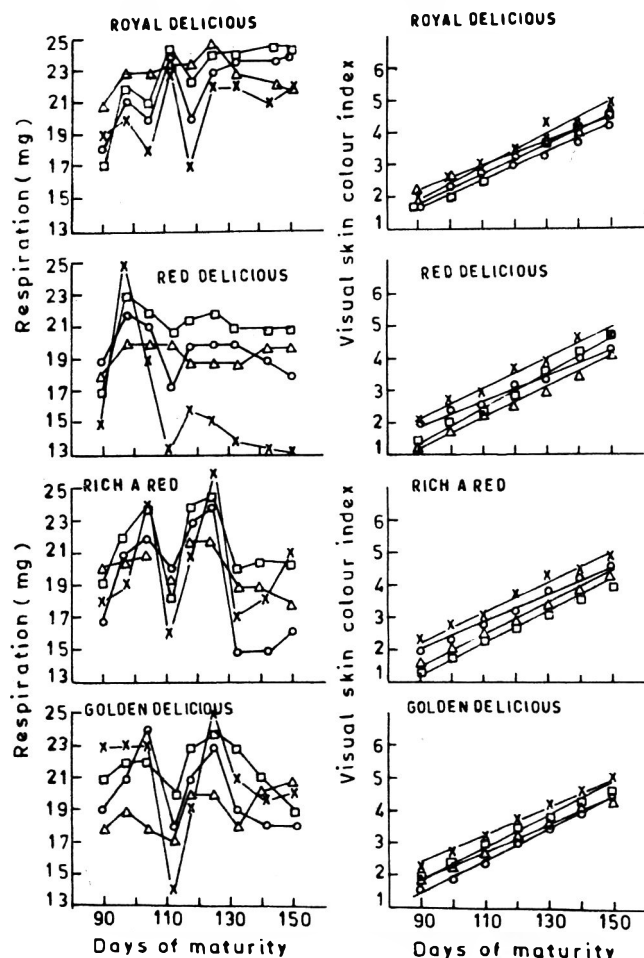


Fig. 1. Variations in respiration rate and visual skin colour index during maturation of apples.
 x—x season 1; o—o season 2; □—□ season 3;
 △—△ season 4.

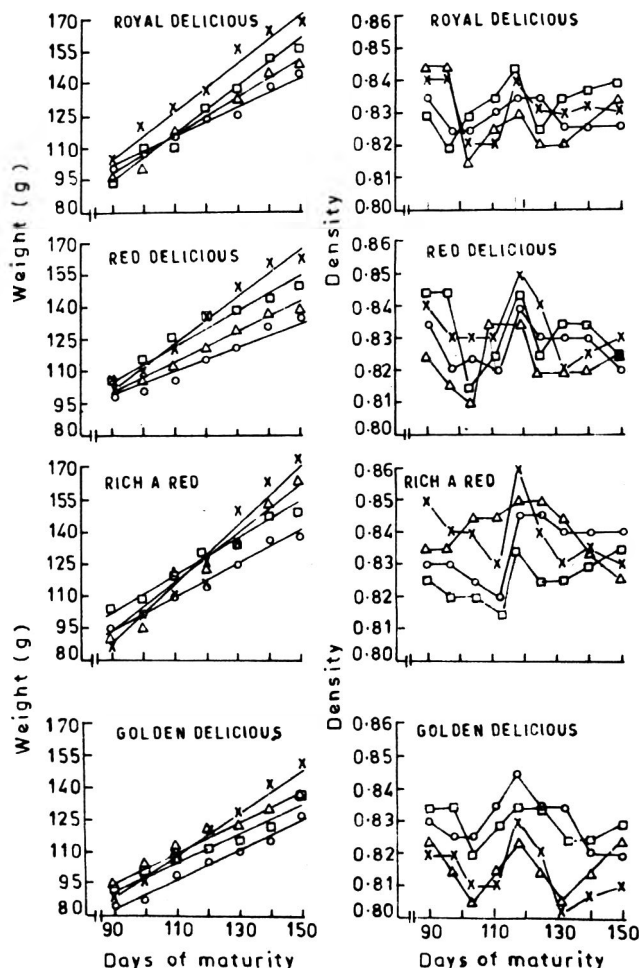


Fig. 2. Variations in weight and density during maturation of apples.
 Legend as in Fig. 1.

as an interval as the fruits fall into different maturity stage distributions as shown above. In other words, the interval between SPI=3.5, SCI=6 and SPI=4.5, SCI=6 in the MLR clearly define the optimum harvest maturity period.

Similarly, MLR equations between SPI, SCI and any one of the other five maturity parameters viz. TSS, acidity, diameter, weight and MT puncture values were determined. The limits for any parameter for optimum maturity were obtained for the two sets of

values (SPI=3.5, SCI=6 and SPI=4.5, SCI=6). In all these, the multiple correlation coefficients are highly significant establishing that the limits worked out are reliable. These limits for optimum maturity are given in Table 3. The overall limits shown for each cultivar accommodating the four years seasonal variations define the optimum maturity. In TSS and acidity, limits for 'Golden Delicious' were higher for the optimum harvest maturity than the other three varieties. In diameter, weight and MT puncture values, these limits were comparable among the varieties.

The study clearly showed that apples must be picked at optimum harvest maturity for better eating quality and storage life. The optimum values determined for TSS, acidity, diameter, weight, and MT puncture values as affected by seasonal variation in four cultivars of Delicious apples will help harvesting of fruits at the correct maturity. Further, the methodology would be useful to study the seasonal, varietal and regional differences in apple quality as also the effect of various pre-harvest and post-harvest treatments on apples.

TABLE 2. PERCENTAGE PROBABLE DISTRIBUTION PATTERNS OF ACCEPTABLE LIMIT OF MATURE FRUITS FOR OPTIMUM HARVEST BASED ON STARCH PATTERN INDEX (SPI)

SPI = 4.0		SPI = 3.75-4.25			SPI = 3.50-4.50			
SIM	M	SOM	SIM	M	SOM	SIM	M	SOM
25	50	25	37.5	50	12.5	50	50	0
12.5	75	12.5	25	75	0	0	50	50
0	100	0	12.5	50	37.5			
			0	75	25			

M - Mature (optimum) SIM - Slightly immature
 SOM - Slightly overmature

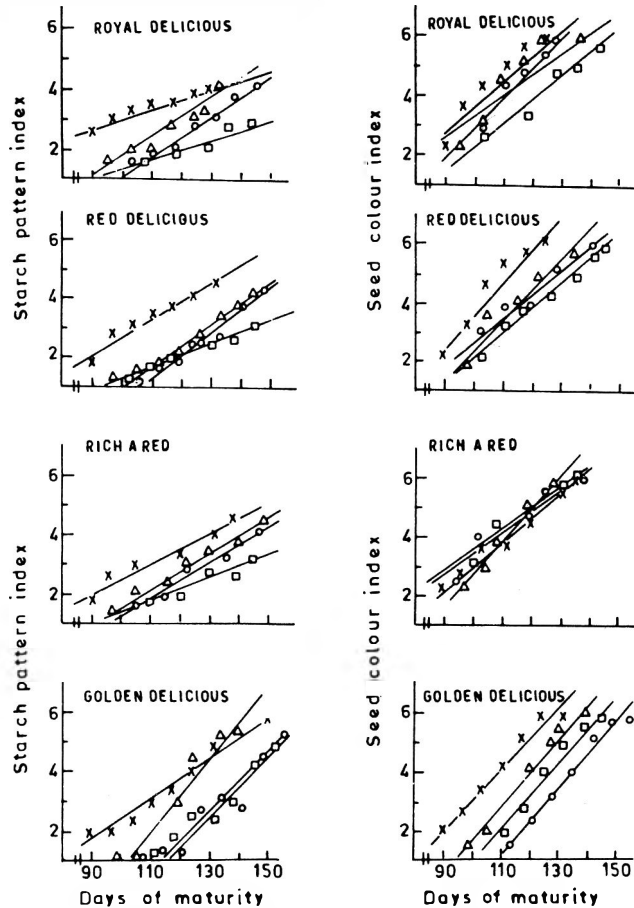
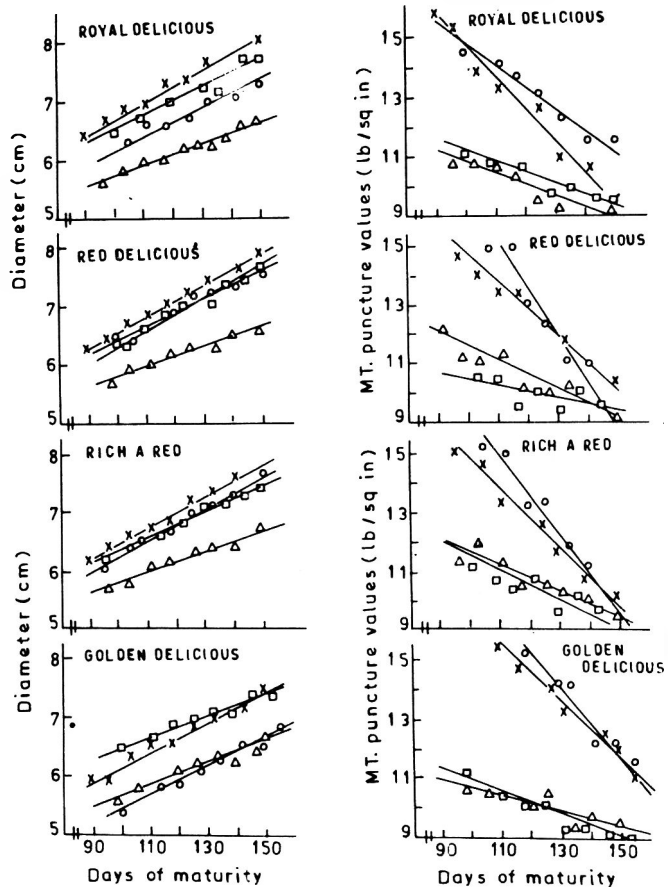


Fig. 3. Variations in diameter and MT puncture values of apples Legend as in Fig. 1.

Fig. 4. Variations in starch protein index and seed colour index during maturation of apples. Legend As in Fig. 1.

TABLE 3. LIMITS FOR DIFFERENT PARAMETERS FOR OPTIMUM MATURITY

Season	Total soluble solids (%)	Acidity (%) (malic acid/100 g)	Diameter (cm)	Weight (g)	Mt puncture values (lb/sq.in.)
<i>Royal Delicious</i>					
1	11.0-11.2	0.17-0.22	7.1-7.6	142.7-174.2	10.4-13.2
2	11.7-11.8	0.26-0.27	6.8-7.0	138.2-150.0	10.8-12.0
3	11.0-11.5	0.24-0.25	7.4-7.6	137.8-156.0	10.2-10.4
4	10.0-10.4	0.15-0.16	6.2-6.4	139.4-162.1	9.8-10.0
	(10.0-11.8)	(0.15-0.27)	(6.2-7.6)	(137.8-174.2)	(9.8-13.2)
<i>Red Delicious</i>					
1	11.2-11.8	0.22-0.23	6.9-7.3	132.8-161.6	11.8-13.0
2	12.4-13.0	0.27-0.28	7.5-7.9	154.6-167.0	10.1-11.2
3	8.6-9.5	0.25-0.28	7.3-7.4	143.7-165.6	10.4-10.8
4	11.2-12.0	0.15-0.16	6.4-6.6	149.1-164.3	8.8-9.5
	(8.6-13.0)	(0.15-0.28)	(6.4-7.9)	(132.8-167.0)	(8.8-13.0)
<i>Rich-a-Red</i>					
1	11.2-11.6	0.24-0.28	7.0-7.6	140.9-169.1	12.0-13.0
2	12.8-13.1	0.30-0.31	7.2-7.4	152.4-166.0	11.0-12.0
3	12.4-13.8	0.25-0.26	7.3-7.6	143.7-167.5	10.1-10.3
4	11.3-12.4	0.16-0.17	6.4-6.6	146.6-169.7	9.6-10.3
	(11.2-13.8)	(0.16-0.31)	(6.4-7.6)	(140.9-169.7)	(9.6-13.0)
<i>Golden Delicious</i>					
1	13.1-13.2	0.42-0.44	6.6-6.9	126.7-138.3	14.2-15.0
2	15.2-16.1	0.46-0.48	6.6-6.8	113.7-116.3	11.8-12.5
3	14.0-14.4	0.45-0.46	7.2-7.4	120.2-127.3	9.0-9.3
4	12.2-12.7	0.40-0.41	6.4-6.5	116.9-132.8	9.8-9.9
	(12.2-16.1)	(0.40-0.48)	(6.4-7.4)	(113.7-138.3)	(9.0-15.0)

Figures in the parenthesis indicate overall limit

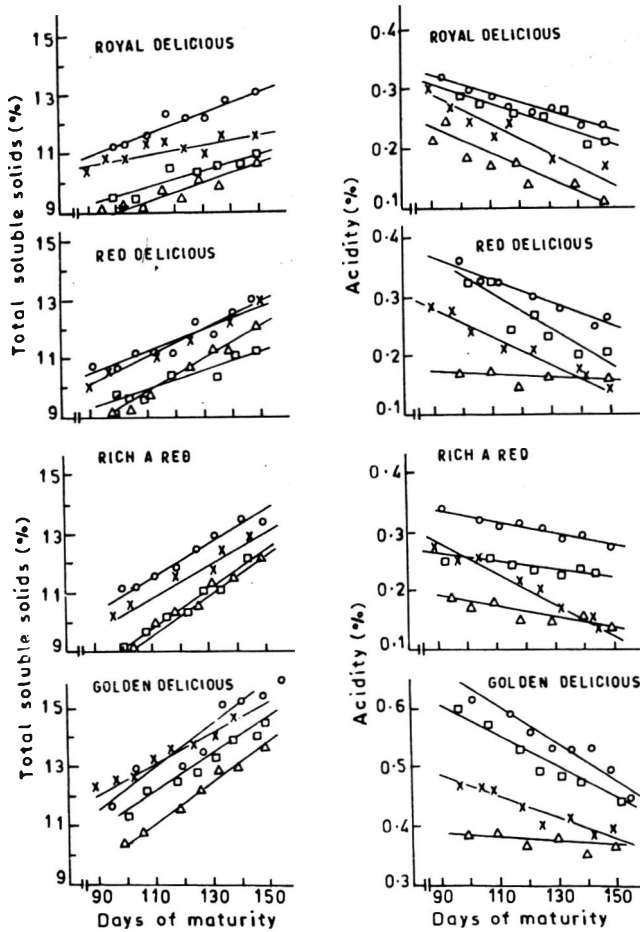


Fig. 5. Variations in total soluble solids and acidity during maturation of apples
Legend as in Fig. 1.

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Studies on the Baking Potential of Non-wheat Composite Flours

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The baking properties of non-wheat flour composites of corn-cassava starch-soybean and corn-cassava starch-cowpea in breadmaking were examined. Results revealed that these non-wheat composite flours do not lend themselves to satisfactory dough development. Their performances were, however, improved when prepared in the form of batter. Consequently, these non-wheat blends required up to 98% water to produce acceptable product. Bread volume increased in corn-cassava-cowpea blend over its corn-cassava-soybean counterpart resulting in lighter loaf. Increasing the cowpea level above 5% lowered the quality of bread. The corn-cassava-cowpea blend was more acceptable than the corn-cassava-soybean sample. The implications of low gluten and protein substitution are discussed.

Bread is traditionally produced from wheat flour and wheat is grown satisfactorily only in some regions of the world. The implication is that non-wheat growing regions must either import their wheat or bread, if they want to eat bread. The other alternative is to exclude bread from their dietary regime. In Nigeria, it is easier for adults than it is for children to substitute another food item for bread. Nigeria's wheat import bill was estimated at over 500 millions in 1985. This no doubt constituted a major financial drain on the country's meagre foreign exchange reserve. The inability of some countries to sustain their wheat import and the need to have bread, now make it imperative that some substitutes for wheat in breadmaking must be found.

The concept of composite flour in breadmaking was introduced many years ago. Graded levels of wheat flour were substituted with non-wheat flours to achieve degrees of success in breadmaking^{1,2}. Indigenous non-wheat flours were utilised in replacing portions of wheat flour in breadmaking³⁻⁶. In all these studies, the composite flours were blends of wheat and non-wheat flours. These investigators assumed that the availability of wheat was assured and sought to reduce the quantity used.

Although news reports claim that bread from non-wheat cereals and even from root tubers had been produced, there is dearth of information in the literature of such break-throughs. In selecting components of the non-wheat composite flours for breadmaking, raw materials that enjoy cultural acceptance should be considered in conjunction with their nutritional potential. This study was, therefore, an attempt to evaluate the baking characteristics of non-wheat composite flours of corn-cassava starch-soybean and corn-cassava starch-cowpea.

Materials and Methods

Cowpea flour was prepared by dry milling the undehulled cowpea with a laboratory mill (Bamford 25 Grinding Mill, Bamford, England) to pass through a sieve of 200 microns. The other already processed ingredients were obtained from local market and the flours reground where necessary to 200 microns.

In one formulation, a composite flour of not less than 60 per cent corn flour, not more than 30 per cent cassava starch, and not more than 10 per cent full-fat soybean flour was used. The percentage of minor ingredients was sugar 4, salt 2, yeasts 2 and fat 1 of total flour. The water varied with composition of composite flour and the straight dough method was used. Another formulation tried was a composite flour of 80 per cent cowpea flour or full fat soybean flour. The percentage of minor ingredients was sugar 8, fat 6, yeast 2, and salt 1.5 of composite flour. Water requirement was variable and batter method was used.

The yeast (Engedura brand) was dissolved in warm water (38°C) at a ratio of 1:5. The dry ingredients were mixed with a Hobart dough mixer at a slow speed for 5 min. The dissolved yeast and remaining water were added to the dry mix and mixed for 10 min at medium speed. The resulting batter was allowed to bulk ferment for 30 min. This was followed by mixing at medium speed for 5 min. The composite batter was scaled and proofed for at least 30 min, and baked at 220°C.

The bread volume was measured with a bread volumeter operating on the principle of seed displacement after the loaves had cooled. The loaves were sensorily evaluated in terms of appearance, crust colour, crumb colour, grain, texture, flavour, and chewability by untrained panelists.

Results and Discussion

The major observation with non-wheat flour was that it did not lend itself to the conventional straight dough method of bread manufacture. This characteristic behaviour of non-wheat flour was independent of composite flour composition. The water requirement of non-wheat flour was higher than the all-wheat control and increased as the corn flour fraction increased. The higher water absorption capacity of corn flour explains the observed higher water requirement. The resulting dough of non-wheat composite flour lacked extensibility and was difficult to handle during mixing and subsequent baking. The lack of extensibility was due to the absence of gluten in the corn-cassava starch-soybean blend. The non-wheat bread was longer than the control for the same loaf size. The rate of heat penetration was obviously slower for non-wheat as revealed by the crumb structure and this was responsible for the longer baking time.

TABLE 1. VOLUME VARIATION OF NON-WHEAT COMPOSITE BREAD
Composite flour type

Corn	Composite flour type		Loaf vol.* (%)
	Cassava Starch	Soybean	
All-wheat (control)	—	—	100
60	30	10	41
70	20	10	33
80	15	5	28
85	10	5	20
100	0	0	17

*Average of three determinations

The loaf volume of the resulting bread from non-wheat composite flour using the straight dough method was unacceptably low (Table 1). Even at 60 per cent corn flour, the loaf volume was less than 50 per cent of the all-wheat control. The results showed that the loaf volume decreased progressively with increased level of corn flour. The all-corn bread presented only 17 per cent of the all-wheat bread volume. The non-wheat products were too heavy indicating dense structure. They were rock-like hard and could not be sliced. The 'loaves' showed extensive cracking; gully formations on the crust were evident. The resulting non-wheat products were generally unacceptable and did not resemble the all-wheat control bread. The results showed that acceptable products could not be produced through the straight dough method of breadmaking.

Results from the experiment using batter instead of the traditional dough method of breadmaking showed greater promise. The samples from the batter method resembled more of bread than those prepared by the dough method in all categories of bread evaluation. The resulting batter product was sliceable, had the shape and appearance of bread, and did not show extensive cracks.

Water requirement substantially increased to 97-100 per cent of the non-wheat composite flour. With the addition of 100 per cent water based on non-wheat composite flour, there was minimal cracking of the crust and better crumb texture than similar samples with 97 per cent water (Table 2). It was evident that

TABLE 2. BREAD CHARACTERISTICS OF NON-WHEAT COMPOSITE FLOURS

Bread sample	Composite flour type				Water (%)	Loaf vol. (cm ³)	Loaf wt. (g)	Sp. loaf vol. (cm ³ /g)	Appearance	Crust colour	Crumb colour	Grain	Texture	Chewability	Flavour
	Corn	Cas-sava starch	Soy-bean	Cow pea											
A	85	10	5	—	97	50	250	2.00	Mod. cracks light	Bright	Bright	Good	Good	Chewy Crumbly	Good
B	85	10	—	5	97	600	275	2.18	Mod. cracks light	Slight dark	Slight dull	Good	Good	Chewy crumbly	Excellent
C	80	10	—	10	98	800	450	1.78	Min. cracks heavy	Dark	Dull	Good	Good	Chewy crumbly	Beany
D	85	10	—	5	100	1000	425	2.35	Min. cracks light	Slight dark	Slight dull	Good	Poor dense	Chewy crumbly	Excellent

the water requirement for the 85 per cent corn flour, 10 per cent cassava starch, and 5 per cent cowpea flour composite was about 100 per cent on flour basis. Corn bread baked with up to 100 per cent water content reportedly resulted in minimally cracked surface. Higher water requirement resulted in lower specific volume of the non-wheat bread. Bread samples B and D (Table 2) were the same in all respects except in their water content. The low specific volume resulted in heavy and structurally dense bread, though less crumbly.

The differences between samples A and B (Table 2) were due to influence of soybean flour and cowpea flour, respectively. There were no significant differences in specific volume. However, the results were substantially different from the specific volume reported for starch or wheat bread. The slightly dark colour of the composite bread containing cowpea resulted from milling cowpea without dehulling. When the cowpea fraction of the composite increased, both crust and crumb colour were negatively affected as indicated in sample C (Table 2).

The flavour of the composite bread containing cowpea flour was more acceptable to the panelists than the counterpart soybean flour. This observation can be traced to traditional food habit. People in this ecological zone traditionally accept cowpea in whatever form it is prepared and are likely to prefer it to the foreign soybean flavour. Although the cowpea flavour is widely accepted in this region, when the content in the composite flour was 10 per cent, the beany flavour was detectable (Table 2). The results showed that incorporation of up to 10 per cent cowpea flour in the non-wheat composite bread negatively affected the bread flavour. The beany flavour problem had been identified as a major factor affecting the utilisation of legumes in food formulations.

The increase in cowpea flour component resulted in low specific loaf volume. It had been reported that increased protein addition in bread caused reduction in loaf volume of substituted wheat protein. This is because of lack of structure-forming proteins. Results of this work indicate that other factors like moisture content, proofing time, and flour composition also contribute to variation in loaf volume. For the non-wheat blends studied, 30 min proofing was found adequate. The bread had flat or dome-shaped top and open crumb texture. When proofing time was extended beyond 30 min the resulting loaf had depressed top, compact crumb and dense texture (Table 3).

Low gluten level of non-wheat flours had been identified as the major cause of poor loaf volume, crumbly texture, and general poor quality of non

TABLE 3. EFFECT OF PROOFING TIME ON QUALITY OF NON-WHEAT COMPOSITE FLOUR BREAD

Loaves	Proofing time (min.)		
	30	35	40
Weight (g)	250	260	290
Volume (cm ³)	850	800	825
Sp vol. (cm ³ /g)	3.40	3.08	2.84
Top appearance	Flat/dome-shaped	Slightly depressed	Depressed
Crumb texture	Open	Slightly compressed	Compact

wheat bread¹¹⁻¹⁴. In all our studies, the non-wheat batter developed good dome-shaped top after proofing. However, the structure collapsed during baking resulting in either flat or depressed top. The inability of the non-wheat batter to retain the dome-shaped structure was due to little or no gluten network formed. The structures formed during proofing were obviously too weak to withstand baking conditions. Consequently, the loaves collapsed during baking. The absence of gluten in non-wheat batter was responsible for the observed poor gas retention which in turn influenced the bread texture.

It is concluded that the incidence of collapsed structure and depressed top in non-wheat breads could be reduced by using narrow pans. Utilising the batter approach, it is possible to produce bread using corn-cassava starch-legume blends; it has a characteristic which is distinct from regular wheat bread. Such special bread should be evaluated on its own merit, if the concept of non-wheat bread is to survive and becomes acceptable.

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Supplementation of Glycerolysed Oils and Alpha-amylases in Breadmaking. I. Effect on Rheological Properties of Dough

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Effect of groundnut oil, glycerolysed groundnut oil, cottonseed oil and glycerolysed cottonseed oil with and without alpha-amylase supplements on rheological properties of dough was evaluated using Brabender Farinograph and amylograph. The treatments had profound effect in decreasing dough consistency after 15 min mixing. However, peak viscosity and viscosity of paste at 95°C were appreciably increased with oils/glycerolysed oils. Mixing tolerance index and gelatinization temperature were significantly decreased. Alpha-amylases lowered falling number values and increased maltose. With intimate mixing of oils/glycerolysed oils, falling number values were found to be increased whereas maltose value decreased. Effect of increasing levels of incorporation of oils/glycerolysed oils is also presented.

The role of surfactants in breadmaking as antistalants has been studied. The use of antistaling additives has been reviewed by Maga¹. Mono- and di-glycerides of fatty acids have been used in breadmaking and their beneficial effect on loaf of high protein bread have been widely accepted². The interaction of the surfactants in medium protein flour loaves have given rise to doubt because of the tendency of the loaf to become crumbly when stored. Glycerolysed oil as an additive in bread making has the advantage of easy and economic dispensation during bread making because of its desirable melting point and flow properties³.

Many workers have suggested that shortenings function as improving agents in bread by acting as lubricants between the starch granules and the gluten filaments^{4,5}. However, very few reports have been published on the rheological and baking properties as affected by oils/fatty acids/glycerinated fats. Incorporation of fatty acids increase the gelatinization viscosity^{6,7}. However, it has been reported by Gray and Schoch⁸ that fatty acids decreased hot paste viscosity. Arya and Narsimha Murthy⁹ observed that triglycerides and vegetable oils did not increase the gelatinization viscosity of wheat *atta*.

Alpha-amylase supplements have limited role to play. They provide a partial solution to counteract the firming of bread after baking. The effect of different alpha-amylase supplements alone on the dough and paste characteristics of Indian wheat flours¹⁰⁻¹² and high protein bread flours of Canadian and U.S. wheats¹³⁻¹⁶ has been reported. There is hardly any report which describes the effect of alpha-amylases along with oils/fatty acids/glycerinated fats on rheological

properties of dough. Therefore, this investigation was undertaken to observe the effect of groundnut oil and cottonseed oil and their glycerolysed preparations with and without alpha-amylase supplements on mixing and pasting characteristics of wheat dough.

Materials and Methods

Flour: 'PBW-12' var. wheat (1983-84) was milled into flour of 74.0 per cent extraction in a Buhler Pneumatic mill (MLU 202). The flour contained 9.7 per cent crude protein and 9.1 per cent damaged starch. It had a diastatic activity of 235 mg maltose/10 g flour and a colour grade of 4.3.

Glycerolysed oils: Glycerolysed groundnut and cottonseed oils were prepared in the laboratory³ and they contained 20.3 and 6.0 per cent alpha-mono-glycerides, respectively, as determined by Standard AOCS¹⁷ procedure.

Amylases: Wheat malt was prepared in the laboratory. Fungal amylase was Novo fungamyl (Denmark) whereas bacterial alpha-amylase was obtained from M/s Schwarz (U.S.A). The amylases were supplemented at a level of 8.0, 20.0 and 0.5 S.K.B./100 g flour, respectively. To find out the optimum level of amylases, preliminary trials were carried out according to the study of Harinder *et al*¹².

Chemical analysis: Protein, diastatic activity and colour grade were determined according to standard AACC¹⁸ procedure.

Rheological studies: AACC¹⁸ method was followed for Brabender farinograph and Brabender amylograph studies. Flour had a farinograph water absorption of 59.8 per cent. It was mixed for 15 min in

a farinograph. Seventy g flour (on 14 per cent moisture basis) was used in the amylograph for observation of pasting properties. The results were average of two replications.

The semi-automatic apparatus Hagberg falling number AB, Sweden, was used and diastatic activity was determined according to AACC¹⁸ procedure. Average results of three replications are being reported.

Results and Discussion

The farinograph curve characteristics as influenced by intimately mixing of oils/glycerolysed oils with and without alpha-amylase supplements are presented in Table 1. The maximum consistency of dough in the farinograph bowl was the most appreciably affected parameter. It decreased on addition of oils/glycerolysed oils. The increase in the level of usage from 0.5 to 1.0 per cent and/or supplementation with alpha-amylases further decreased both characteristics. Maximum consistency observed was 435 to 500 B.U. The drop in consistency after 15 min of mixing in the farinograph ranged from 55 to 110 B.U. Consistency after 15 min. mixing was significantly decreased with the addition of amylases and also with the increased level of oil/glycerolysed oil (Table 2).

Alpha-amylases played a significant role in reducing the dough development time (Table 3). However, no definite interaction was observed when used in combination with oil/glycerolysed oil. Mixing tolerance index was adversely affected by alpha-amylases and oils/glycerolysed oils. The increase in the level of incorporation from 0.5 to 1.0 per cent of oil/glycerolysed oil further lowered the mixing tolerance index. The decrease ranged from 10 to 40 B.U., the maximal decrease being with the addition of 1 per cent glycerolysed cottonseed oil. The effect of glycerolysed groundnut oil on farinograph curve characteristics was more pronounced than that of glycerolysed cottonseed oil. This is attributed to the higher amount of alpha-monoglycerides in glycerolysed groundnut oil. Garti *et al*¹⁹ reported negligible effect on mixing tolerance on addition of 0.5 per cent mono-and diglycerides to flour. However, according to Tsen and Weber²⁰ developing time of dough was decreased when monoglycerides were used at 0.5 per cent level in the dough of high protein Canadian bread wheat flour. Dough properties are deleteriously affected by the level and nature of alpha-amylase supplements as studied with farinograph¹⁰⁻¹².

The results showing the effect of oils/glycerolysed

TABLE 1. EFFECT OF OILS AND GLYCEROLYSED OILS WITH AND WITHOUT ALPHA-AMYLASE SUPPLEMENTS ON MAXIMUM CONSISTENCY AND CONSISTENCY AFTER 15 MIN MIXING

Name	Oil/glycerolysed oil Quantity (g/100g)	Max consistency, B.U.				Consistency after 15 min, B.U.			
		Control	Wheat malt	Fungamyl	Bacterial	Control	Wheat malt	Fungamyl	Bacterial
Control	0.0	500	500	480	495	415	415	370	405
GO	0.5	470	470	460	455	395	395	360	370
	1.0	460	450	435	440	385	385	355	355
GGO	0.5	460	450	450	455	395	380	390	385
	1.0	435	430	430	430	375	370	385	375
CO	0.5	470	490	460	455	375	395	360	395
	1.0	460	460	430	450	385	380	350	385
GCO	0.5	470	460	460	470	395	380	370	395
	1.0	455	435	435	455	385	370	355	385

GO, ground nut oil; GGO, glycerolysed groundnut oil; CO, cotton seed oil; GCO, glycerolysed cottonseed oil

TABLE 2. ANALYSIS OF VARIANCE FOR VARIOUS RHEOLOGICAL CHARACTERISTICS OF DOUGH

Source of variation	D.F.	Mean sum of squares									
		Max consis- tency	Consist- ency after 15 min mixing	Dough develop- ment time	Mixing toler- ance Index	Gela- tini- zation temp.	Peak visco- temp	Peak visco- sity	Viscosity at 95°C	Falling No.	Diasta- tic acti- vity
α-amylases	3	636.6**	2039.4**	2.00**	131.3*	0.40*	141.43**	575460.2**	388270.6**	33664.1**	10750.3**
Oils/gly. oils	3	282.4**	43.6	0.37	145.1*	0.97**	12.47**	48419.9*	18263.5**	1386.7**	293.7**
Levels	2	10420.3**	3113.0**	0.95**	1602.1**	0.30	2.40	39325.7	22243.9**	1800.5**	883.4**
Error	39	52.82	111.2**	0.16	38.8	0.14	1.95**	13272.7**	2053.6**	118.5**	23.4

TABLE 3. EFFECT OF OILS AND GLYCEROLYSED OILS WITH AND WITHOUT ALPHA-AMYLASE SUPPLEMENTS ON DOUGH DEVELOPMENT TIME AND MIXING TOLERANCE INDEX

Oil/glycerolysed oil		Dough development time. min				Mixing tolerance index, B.U.			
Name	Quantity (g/100g)	Control	Wheat malt	Fungamyl	Bacterial	Control	Wheat malt	Fungamyl	Bacterial
Control	0.0	3.8	3.1	2.5	3.6	90	90	75	75
GO	0.5	3.5	3.8	3.8	4.0	75	80	80	75
	1.0	3.5	3.5	3.8	4.3	70	70	70	65
GGO	0.5	3.4	3.6	3.1	4.0	65	70	70	70
	1.0	4.1	4.5	2.5	5.0	60	60	45	65
CO	0.5	3.5	3.5	3.5	3.6	75	80	80	60
	1.0	3.5	3.5	3.5	3.0	65	70	70	60
GCO	0.5	3.8	3.1	2.5	3.5	65	65	70	70
	1.0	4.5	3.9	2.5	4.1	50	60	55	65

TABLE 4. EFFECT OF OILS AND GLYCEROLYSED OILS WITH AND WITHOUT ALPHA-AMYLASE SUPPLEMENTS ON GELATINIZATION TEMPERATURE AND PEAK VISCOSITY TEMPERATURE

Oil/glycerolysed oil		Gelatinization temp. (°C)				Peak viscosity temp. (°C)			
Name	Quantity (g/100g)	Control	Wheat malt	Fungamyl	Bacterial	Control	Wheat malt	Fungamyl	Bacterial
Control	0.0	60.2	59.5	60.2	60.0	86.0	78.5	86.5	87.7
GO	0.5	60.0	59.5	60.2	59.5	86.5	77.5	86.5	85.7
	1.0	60.0	59.5	60.2	59.5	87.0	79.5	86.5	84.5
GGO	0.5	61.5	60.0	61.0	60.5	87.5	85.0	88.7	87.2
	1.0	61.0	61.0	61.0	60.2	88.0	86.5	88.5	88.5
CO	0.5	60.5	60.5	60.2	60.2	87.2	79.7	86.5	86.2
	1.0	59.5	60.2	60.2	60.0	86.5	80.5	87.0	86.0
GCO	0.5	60.0	60.2	59.5	60.2	86.0	78.2	86.5	85.7
	1.0	60.2	60.0	59.5	60.0	87.0	78.0	87.2	86.0

oils with and without alpha-amylase supplements on paste characteristics are presented in Tables 4 and 5. The flour-water slurry started thickening between the temperature range of 59.5 and 61.5°C. Peak viscosity was attained at 77.5 to 88.7°C which has been found to be significantly affected by the source of alpha-amylase and oil/glycerolysed oil. The main differences in peak viscosity temperature in the presence of 0 to 1.0 per cent oil/glycerolysed oil in flour water system depended on the source of alpha-amylase supplement. The wheat malt significantly (Table 2) reduced the

temperature at which the flour paste became viscous. Peak viscosity temperature of paste dropped from 86.0 to 78.5 in the presence of 8.0 SKB wheat malt. However, temperature was increased to 85.0 and 86.5°C in the presence of 0.5 and 1.0 per cent glycerolysed groundnut oil, respectively. Type of oil/glycerolysed oil significantly increased the peak viscosity temperature. However, their increasing amounts did not show any effect as evidenced from Tables 2 and 4.

Peak viscosity and viscosity at 95°C were affected

TABLE 5. EFFECT OF OILS AND GLYCEROLYSED OILS WITH AND WITHOUT ALPHA-AMYLASE SUPPLEMENTS ON PEAK VISCOSITY AND VISCOSITY AT 95°C

Oil/glycerolysed oil		Peak viscosity, A.U.				Viscosity at 95°C, A.U.			
Name	Quantity (g/100g)	Control	Wheat malt	Fungamyl	Bacterial	Control	Wheat malt	Fungamyl	Bacterial
Control	0.0	640	175	635	540	360	20	360	270
GO	0.5	650	175	645	570	395	10	380	300
	1.0	670	182	665	595	390	20	378	310
GGO	0.5	850	220	840	822	460	22	450	410
	1.0	915	280	905	902	590	80	580	565
CO	0.5	642	175	635	558	375	20	360	305
	1.0	675	190	650	570	390	25	380	305
GCO	0.5	700	180	682	650	390	12	382	382
	1.0	725	190	710	695	430	20	385	385

most with the supplementation of alpha-amylases as well as oils/glycerolysed oils. Alpha-amylases decreased the peak viscosity whereas oils/glycerolysed oils increased it (Table 5). The wheat malt alpha-amylase decreased the peak viscosity value from 640 to 175 A.U. Maninder and Bains¹¹ reported a decrease of 67.9 to 69.5 per cent in the peak viscosities when barley and wheat malt supplements were added to the wheat flour. Starches of different origin showed differences in the peak viscosities as reported by Medcalf *et al*²¹. Hutchinson²² observed substantial differences in the four paste viscosities which were unexplained by the variations in alpha-amylase activity. Glycerolysed groundnut oil notably increased the peak viscosity of control from 640 to 850 and 915 A.U. on account of 0.5 and 1.0 per cent supplementation, respectively. The increase in paste viscosity was more conspicuous in fungamyl and bacterial alpha-amylase. Glycerolysed cottonseed oil increased the paste viscosity to 700 and 725 A.U. (from 640) with 0.5 and 1.0 per cent level of incorporation respectively. Collison²³ reported that surfactant is adsorbed on the surface of the starch granules and thereby decreases the swelling power and gelatinization viscosity. However, in the present study glycerolysed oils significantly increased the paste viscosities. It is likely that an emulsifier having hydrophilic as well as hydrophobic moieties complex with amylose of the starch granule resulting in inter-granular adhesion. Such complexes show high paste viscosities.

Viscosity of pastes at 95°C in the amylograph was found to be increased when oils/glycerolysed oils were used. Viscosity increased from 360 to 395 and 390 A.U. with groundnut oil and 460 and 490 A.U. with glycerolysed groundnut oil at a level of 0.5 and 1.0 per cent, respectively. However, increase in viscosity with the use of cottonseed oil at 0.5 and 1.00 per cent was only 15 and 30 A.U. respectively. Wheat malt pastes

irrespective of addition of oil/glycerolysed oil exhibited extremely low paste viscosities at 95°C as compared to higher paste viscosities when fungal and bacterial alpha-amylases were used with/without oil/glycerolysed oil. This can be attributed to the extensive and drastic dextrinization of starch even at very low levels of supplementation of wheat malt¹². The results of different alpha-amylase supplements on paste characteristics are in accordance with the results of Johnson and Miller¹³, Amos¹⁴, Pomeranz and Shellenberger¹⁵, Mamaril¹⁶ *et al*, Maninder¹⁰ and Maninder and Bains¹¹. However, there is no report which simultaneously compared both types of supplements i.e. alpha-amylases and oil/glycerolysed oil.

Wheat malt supplement notably reduced the falling number values of the flour followed by bacterial and fungal amylases (Table 6). The present data are in agreement with the reports of Maninder¹⁰ and Harinder *et al*¹². Effect of groundnut as well as cottonseed oils on falling number values was negligible. Unlike these, glycerolysed preparation tended to increase falling number values of the control as well as of the samples containing alpha-amylases. Further, increase in the level of incorporation of glycerolysed oils increased the falling number values; there was a marked increase in case of fungamyl and bacterial amylase supplemented flour. Selvaraj *et al*²⁴ reported that incorporation of 0.5 per cent glyceryl monostearate increased the falling number value of flour from rain damaged wheat from 152 to 162 sec.

There is a definite effect of alpha-amylases in increasing the diastatic value of flour^{10-12,25-27}. However, diastatic activity was lowered when the flour was intimately mixed with glycerolysed oils (Table 6). It was further decreased as the level of glycerolysed preparation was increased. On comparing the diastatic values of the samples in which glycerolysed groundnut oil was used, the decrease was more than the

TABLE 6. EFFECT OF OILS AND GLYCEROLYSED OILS WITH AND WITHOUT ALPHA-AMYLASE SUPPLEMENTS ON HAGBERG FALLING NUMBER AND DIASTATIC ACTIVITY

Oil/glycerolysed oil	Quantity (g/100g)	Hagberg falling No. (sec)				Diastatic activity (mg maltose/10g)			
		Control	Wheat malt	Fungamyl	Bacterial	Control	Wheat malt	Fungamyl	Bacterial
Control	0.0	464	345	435	416	235	305	268	261
GO	0.5	465	346	437	419	223	299	261	261
	1.0	467	355	444	425	223	296	255	258
GGO	0.5	485	350	475	465	219	292	249	249
	1.0	497	368	486	467	199	279	235	242
CO	0.5	463	345	435	416	226	299	267	261
	1.0	466	352	440	420	226	296	267	258
GCO	0.5	474	353	465	462	223	299	258	255
	1.0	487	357	479	466	216	292	252	245

corresponding samples having glycerolysed cottonseed oil. This difference may be attributed to the interaction of alpha-amylase supplements and glycerolysed preparations, especially glycerolysed groundnut oil which had a higher alpha-monoglyceride content.

The results of the present investigation conclusively demonstrate that glycerolysed groundnut and cottonseed oils did produce beneficial effect on paste characteristics relating to fungal and bacterial amylases but not to the same extent as obtained with cereal amylase. They had a definite interaction to check the influence of alpha-amylases on dough characteristics.

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Supplementation of Glycerolysed Oils and Alpha-Amylases in Bread Making. II. Effect on Baking Quality and Firmness of Bread

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The effect of incorporation of glycerolysed groundnut oil/cottonseed oil and/or optimal alpha-amylases on baking quality was investigated. Dough handling properties were found to be improved when glycerolysed oils were supplemented along with alpha-amylases. Loaf volume was increased in control from 1430 to 1570 ml when 0.5 per cent glycerolysed groundnut oil was used along with 8.0 SKB cereal amylase. Texture of the loaves was substantially improved by the glycerolysed oils. Incorporation of 0.5 per cent glycerolysed groundnut oil distinguishably imparted a softer texture to the loaves but they turned crumbly after storage for 72 hr. Bread made with 0.25 per cent glycerolysed oils showed no such tendency to crumbliness. Storage had a profound effect on firmness of bread.

It has been reported that incorporation of monoglycerides/glyceryl monostearate/superglycerinated fats during breadmaking improves loaf volume and crumb texture of bread¹⁻⁸. Improving effect of alpha-amylases on loaf volume has also been reported by various workers⁹⁻¹². Improvement in sensory quality of bread has also been ascribed to alpha-amylase supplements. Thus, in continuation to our previous communication¹³ the effect of supplementation of glycerolysed oils and alpha-amylases on baking quality of bread was undertaken.

Materials and Methods

Flour from 'PBW-12' var. wheat, amylases and glycerolysed oils were same as used in the previous study¹³. Compressed yeast and cane sugar were obtained from the local market. Salt was LR grade from BDH.

Baking: Straight dough remix method of bread making simulating commercial 400 g loaf was followed. Baking formula included the following ingredients: flour (14 per cent m.b.) 250 g; yeast 5.62 g; salt 3.75 g; sugar 5.0 g and potassium bromate 10 p.p.m. The following baking schedule was adopted: mixing-4 min; fermentation-60 min at 30°C; proofing-55 min; baking-25 min at 232°C.

Loaf volume: Loaf volume was determined according to AACC¹⁴ method.

Instron evaluation of bread texture: The Instron Universal Testing Instrument Table model 1111 with the following settings was used to determine the texture of the loaves by compression: Bread slice-12 mm

thick; Drive speed-100 mm/min; chart speed-200 mm/min; force range-2000 g full scale; compression-6 mm.

Firmness of the loaf was measured in g force required for 6 mm compression of the slice at the centre. Softness index was calculated by dividing the force (g) required for the test sample by that for the control.

Results and Discussion

Dough handling: The bake absorption in all cases was about the same as the farinograph absorption of flour i.e. 59.8 per cent. The dough properties of control were satisfactory, whereas those of doughs having the alpha-amylase supplements/oils were rated as good as those of doughs with glycerolysed oils and fungamyl or bacterial alpha-amylase. The dough properties with wheat malt and glycerolysed oils were rated as very good. The dough having wheat malt was light and fluffy to handle.

Loaf volume: It could be seen from Table 1 that loaf volume was significantly affected with oil/glycerolysed oil (GO) and alpha amylases. It was considerably increased by malt and fungamyl as compared to a nominal increase caused by bacterial alpha-amylase. There was more favourable effect on supplementing wheat malt with glycerolysed groundnut oil (GGO), considering the increase in loaf volume which in the case of glycerolysed cottonseed oil (GGO) was about the same as that of control. The present data confirm the earlier results of improvement of loaf volume by glycerinated fats, products of monoglycerides and alpha-amylases^{1,4,6,15,16}. The mechanism of the improving effect of shortening and

TABLE 1. EFFECT OF OILS, GLYCEROLYSED OILS AND ALPHA-AMYLASE SUPPLEMENTS ON LOAF VOLUME (ML) OF PBW-12 LOAVES

Alpha-amylase SKB/100 g	Control	GO	GGO	CO	GCO	
	0.0%	0.5%	0.5%	0.5%	0.5%	0.5%
Control (nil)	0.0	1430	1400	1420	1380	1380
Wheat malt	8.0	1530	1460	1570	1410	1435
Fungamyl	20.0	1525	1450	1540	1400	1445
Bacterial	0.5	1465	1405	1430	1385	1410

Analysis of variance		
Source of variation	D.F.	MSS
Oils/glycerolysed oils	4	7466.9**
Alpha-amylases	3	7568.3**
Error	12	636.0

GO: Glycerolysed oil; GGO: Glycerolysed groundnut oil;
CO: Cottonseed oil; GCO: Glycerolysed cottonseed oil.

TABLE 3. EFFECT OF GLYCEROLYSED OILS, ALPHA-AMYLASE SUPPLEMENTS AND STORAGE ON THE SLICING CHARACTERISTICS OF BREAD

Glycerolysed oil (g/100 g)	Storage (hr)	Slicing characteristics*			
		Control	Wheat malt	Fung- amyl	Bacte- rial
Control (0.0)	3	S	S	S	S
	72	S	S	S	S
GGO (0.5)	3	S	S	S	S
	72	C	LC	LC	S
GCO (0.5)	3	S	S	S	S
	72	C	LC	S	S

S, Satisfactory; C, Crumbly; CL, Less crumbly
Rating of loaves with GO/CO with and without alpha-amylases is same as of control both at 3 and 72 hr of storage.

TABLE 2. EFFECT OF OILS, GLYCEROLYSED OILS AND ALPHA-AMYLASE SUPPLEMENTS ON THE PHYSICAL CHARACTERISTICS OF PBW-12 LOAVES

Alpha-amylase (SKB/100 g)	Oil/GO (g/100 g)	GO		GGO		CO		GCO		
		Crust	Exterior	Crust	Exterior	Crust	Exterior	Crust	Exterior	
Control	0.0	0.0	LB	A	LB	A	LB	A	LB	A
		0.5	PB	A	PB	A	PB	A	LB	A
Wheat malt	8.0	0.0	B	B	B	B	B	B	DB	B
		0.5	B	LB	DB	B	LB	B	B	B
Fungamyl	20.0	0.0	B	B	B	B	LB	LB	B	B
		0.5	B	LB	DB	B	PB	LB	DB	B
Bacterial	0.5	0.0	B	B	B	LB	LB	LB	B	LB
		0.5	LB	LB	B	LB	PB	PB	B	LB

LB, Light brown; PB, Pale brown; B, brown; DB, Dark brown
A, Anaemic; LB, Light brown; B, brown; DB, Dark brown

certain surfactants on loaf volume has been associated with the more expandable dough resulting in higher loaf volume¹⁷.

Physical characteristics of loaves: Crust of the loaves prepared with 0.5 per cent glycerolysed oils and wheat malt/fungamyl was dark brown as compared to the brown crust of bacterial amylase supplemented loaves (Table 2). Exterior of the loaves was improved from anaemic to light brown or brown on incorporation of optimal levels of wheat malt or fungamyl with/without glycerolysed oil. The crumb texture was subjectively rated as "very soft" where glycerolysed oils with alpha-amylase supplements was used as compared to "soft" rating in case of loaves which were supplemented only with oils/glycerolysed oils.

Effect of storage on slicing properties: The slicing properties of the loaves as influenced by glycerolysed oils, alpha-amylase supplements and storage are presented in Table 3. Cooling to room temperature for 3 hr after removal from the oven, ensured satisfactory slicing of the bread. The loaf made with glycerolysed

groundnut oil tended to be highly crumbly when kept for 72 hr. In this respect, the stored control loaf without any additive showed satisfactory slicing as that of alpha-amylase supplemented loaves without any glycerolysed oil. The tendency to crumbliness was less when malt and fungamyl enzymes were used in conjunction with the glycerolysed groundnut oil. Bacterial alpha-amylase supplemented loaves with/without glycerolysed oil showed satisfactory slicing characteristics even after 72 hr of storage.

Effect on firmness of bread: Results showing the effect of glycerolysed oils with and without alpha-amylases on the firmness of bread kept for 8 and 36 hr at 32°C are presented in Table 4. The force required to compress the slice of control bread kept for 8 hr after baking exceeded that of the rest of the test loaves. The minimal force of 160 to 200 g was required to compress the slices of bread made with glycerolysed groundnut oil with bacterial and wheat malt alpha-amylase, respectively. The firmness of all the loaves increased significantly during 36 hr storage at room temperature. The softness index reflected the

TABLE 4. EFFECT OF OILS, GLYCEROLYSED OILS AND ALPHA-AMYLASES ON FIRMNESS OF BREAD CRUMB

Oil/glycerolysed oil (g/100 g)	Storage time (hr)	Firmness, g force for 6 mm compression				Softness index			
		Control	Wheat malt	Funga- amyl	Bacte- rial	Control	Wheat malt	Fung- amyl	Bacter- ial
Control (nil)	8	280	245	250	250	1.00	0.88	0.89	0.89
	36	390	370	370	360	1.00	0.95	0.95	0.92
GO (0.25)	8	275	235	240	245	0.98	0.84	0.85	0.88
	36	380	360	370	355	0.97	0.92	0.95	0.91
GGO (0.25)	8	260	200	220	160	0.93	0.71	0.79	0.57
	36	360	330	345	340	0.92	0.85	0.88	0.87
CO (0.25)	8	280	270	275	275	1.00	0.96	0.98	0.98
	36	380	380	385	375	0.97	0.97	0.99	0.96
GCO (0.25)	8	275	270	270	270	0.98	0.96	0.96	0.96
	36	380	380	380	370	0.97	0.97	0.97	0.95
Analysis of variance		Firmness	Softness						
Source of variation	D.F.	MSS	MSS						
Alpha-amylases	3	1307.3**	0.0133**						
Oils/glycerolysed oils	4	3270.9**	0.030**						
Storage	1	133980.6**	0.010*						
Error	31	180.3	0.0028						

degree of softness of the loaves of different treatments in relation to that of the control. The results are in accordance with the report of Kamel *et al*¹⁸.

The results of the present investigation show good relationship between the force (g) required to compress a slice of bread and the subjective assessment of freshness. On the basis of softness index values, use of glycerolysed oils in conjunction with alpha-amylases was superior to either of the additives alone.

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Mechanical Kneading of *Chhana* and Quality of *Rasogolla*

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Chhana is an acid coagulated product of milk. Kneading, the important step in *rasogolla* making, was attempted with a disc grinder at 22, 47, 72 and 89 cm/sec. disc speeds. Tests on *chhana* had shown that the values of mixing index, flow behaviour index (n), consistency coefficient (K) and time, (T_c) required for deforming a *chhana* sample under a constant pressure became nearly constant when the disc speed was 89 cm/sec. The values of n , K and T_c for the *chhana* obtained at this speed were respectively 0.345, 368 Pa.sⁿ and 24.8 sec. and the corresponding values of a hand kneaded *chhana* used for *rasogolla* making were 0.335, 356 Pa.sⁿ and 15.3 sec. *Rasogolla* was prepared by cooking 10 g ball of kneaded *chhana* in boiling water and later sweetening it in a sugar syrup. The *rasogolla* made from the *chhana* obtained at 89 cm/sec. disc speed showed the minimum deviation in creep behaviour in comparison to the 'market *rasogolla*'. The overall sensory score of the former *rasogolla* differed by 7.5% from the 'market *rasogolla*'.

Chhana is an acid-coagulated product of milk. *Rasogolla* is prepared by cooking balls of kneaded *chhana* in boiling sugar syrup. Studies¹⁻³ have shown that flavour and mouthfeel qualities of *rasogolla* are affected by the type of *chhana* and its moisture content, the degree of kneading, concentration of sugar syrup and the time of cooking. The yield and quality of *chhana* are dependent on type of milk (cow or buffalo) and its composition (mainly fat and casein), temperature of milk at the time of curdling, type of coagulant, pH of coagulation and duration of acidification³⁻⁵.

Kneading is an important step in *rasogolla* making. It is a mixing process in which the rheological and textural properties of *chhana* are changed and the textural properties of *rasogolla* are subsequently affected.

Traditionally the kneading of *chhana* is done manually. For continuous production of *rasogolla*, *chhana* must be kneaded mechanically and for this, a disc grinder can serve the purpose. This paper describes the use of a disc grinder and the quality of *chhana* and *rasogolla* obtained from the grinder. A comparison between hand-kneaded *chhana* and *rasogolla* has been made with the mechanically kneaded *chhana* and *rasogolla*.

Materials and Methods

Preparation of *rasogolla*: Fresh cow's milk collected from a local milkman was used for making *chhana* and preparation of *rasogolla*. The milk was standardized to 4.1 per cent fat and 8.9 per cent S.N.F. *Chhana* was prepared by the method described by Bhattacharya

and Des Raj³. The milk was first heated to boiling and cooled down to 72-74°C. Two per cent citric acid solution at a rate of 100 to 125 ml per litre of milk was added to it. When the coagulation was complete (pH 5.0 to 5.5), the whey was drained through a muslin cloth. The *chhana* was then dipped in tap water for 20-25 min for cooling and partial removal of coagulum. It was then manually squeezed. The moisture content of *chhana* thus obtained ranged between 55 and 58 per cent.

The *chhana* was kneaded in a disc grinder. Ten gram lumps of kneaded *chhana* were made into balls by rotating between palms for about 1 min. Care was taken to see that there were no cracks on the surface of the balls. The *rasogolla* was made by cooking the balls in boiling water for 25-30 min. This *rasogolla* was sweetened by keeping it in sugar syrup (57.5°B_x) for 24 hr.

The mechanically kneaded *chhana* was compared with hand kneaded *chhana* (moisture content 60 per cent) available with a local sweetmeat shop which had the reputation of preparing quality *rasogolla*. This hand kneaded *chhana* will be called here as the 'market *chhana*' and the *rasogolla* made from it as the 'market *rasogolla*'. The sugar syrup used to keep the market *rasogolla* was of 57.5° B_x.

The disc grinder: Fig. 1 shows the disc grinder used in the experiment. The rotating disc 3 is an integral part of the feed auger 1. By tightening the screw 4, the rotating disc and the feed auger could be moved to the right and a pressure applied on the stationary disc 2. Rotation of the feed auger moved the *chhana* mass to the left of the feed hopper and

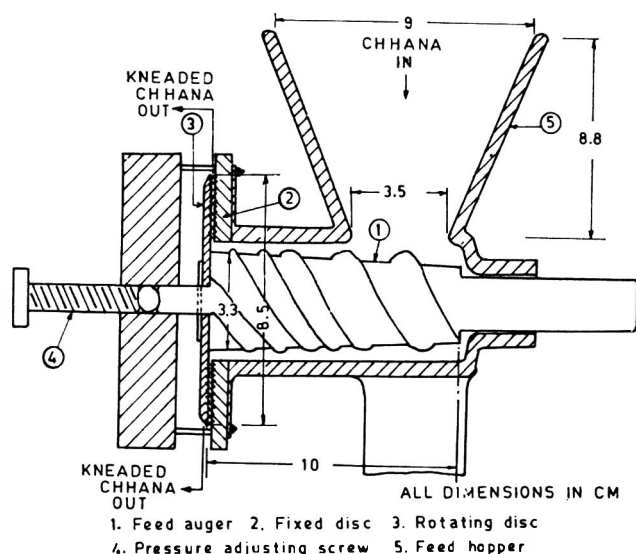


Fig. 1. Disc Grinder

forced it to come out through the space between two discs. The rotation of the feed auger was varied by a 1 hp variable speed d.c. motor.

The primary variable: In the disc grinder, the kneading of *chhana* starts at the feed end of the screw and continues until the *chhana* leaves the periphery of the discs. The degree of kneading is primarily affected by the rate at which the *chhana* mass is sheared between the discs. The rate of shear is the ratio of the peripheral velocity of the rotating disc and the distance between the discs. This rate of shear continuously increases as the *chhana* moves radially outward along the surface of the discs and its value is maximum at the outer periphery of the discs. Since the distance between the two discs of the grinder remains negligibly small during the grinding operation, the outer peripheral speed of the rotating disc was considered as the primary variable in the kneading operation. Four peripheral speeds, viz. 22.25, 46.73, 71.2 and 89.0 were used in the experiment.

Measures of kneading effect: The effect of kneading was evaluated from (1) degree of mixing of *chhana*, (2), rheological properties of *chhana*, (3) creep test on *chhana*, (4) creep test on *rasogolla*, (5) temperature rise of *chhana* balls during cooking, (6) expansion of *chhana* balls during cooking, and (7) sensory qualities of *rasogolla*.

Degree of mixing: The kneading of *chhana* can be considered as a mixing process where a uniformity of distribution of water and solid within the *chhana* mass takes place. The degree of this uniformity achieved was measured by adding common salt (0.3 g salt in 30 g *chhana*) before kneading and measuring standard deviation of electrical conductivity of diluted *chhana* samples (1 g *chhana* in 20 ml water) after the kneading process. A 'modified mixing index', M was used to

express the effectiveness of the mixing process. The value of M is given by⁶

$$M = \sigma / \sigma_0 \quad \dots(1)$$

where, σ is the standard deviation of electrical conductivities of *chhana* samples after kneading, $m\sigma_0$ and σ_0 is the standard deviation of salt content before kneading, g salt/g *chhana*.

The value of σ_0 is expressed as⁶

$$\sigma_0 = \sqrt{\mu(1-\mu)} \quad \dots(2)$$

where, μ is the fraction of salt in *chhana* before kneading. In the present experiment $\mu = 0.3/30 = 0.01$ and from Eqn. (2) $\sigma_0 = 0.0995$.

A Toshniwal Conductivity Bridge (Model CL01/024) was used for the measurement of the electrical conductivity. Five to seven 1-g samples of kneaded *chhana* were taken for finding the value of σ .

Rheological properties of *chhana*: It was assumed that *chhana* would behave as a viscoelastic fluid and the kneading would change its flow behaviour. Since the flow behaviour of a viscoelastic fluid is normally expressed by its consistency coefficient, K and flow behaviour index, n, a rotational viscometer (Brookfield, model: RVT) was used to find these parameters. The instrument measures apparent viscosity, η_a which is related to K and n as⁷

$$\eta_a = (2\omega)^{n-1} K (1/n)^n \quad \dots(3)$$

where, ω is the angular speed of the rotor of the viscometer.

The smallest rotor (No. 7) of the viscometer was used to measure the value of η_a . Several values of η_a were obtained by varying the speed of this rotor. A plot of $\log \eta_a$ vs $\log 2\omega$ yielded a straight line and the values of K and n were calculated from the slope and intercept of this line. When the value of η_a was in Pa.s and that of ω in s^{-1} the value of K was obtained in Pa.sⁿ. Rheological properties of both 'market *chhana*' and the mechanically kneaded *chhana* were evaluated.

Creep test on *chhana*: In this test, a cylindrical piece of *chhana* (10 mm diameter, 12 mm long) was subjected to a constant pressure (102 g/cm² applied on the flat surface of the cylinder) and the time T_C required to compress it by 25 per cent (i.e. to a final height of 9 mm) was measured with the help of the instrument shown in Fig. 2. It was considered that this time would characterise some of the textural properties (e.g. hardness, chewiness, springiness etc.) of *chhana*. The values of T_C reported elsewhere in the paper are the average of three observations.

The instrument (Fig. 2) was developed from the skeleton of a viscometer. The spindle 5 of the instrument could be moved downward by placing weight on the weigh pan 6 and by rotating the cam 9. The downward movement of the spindle caused a movement of the needle on dial 2. The marks on the

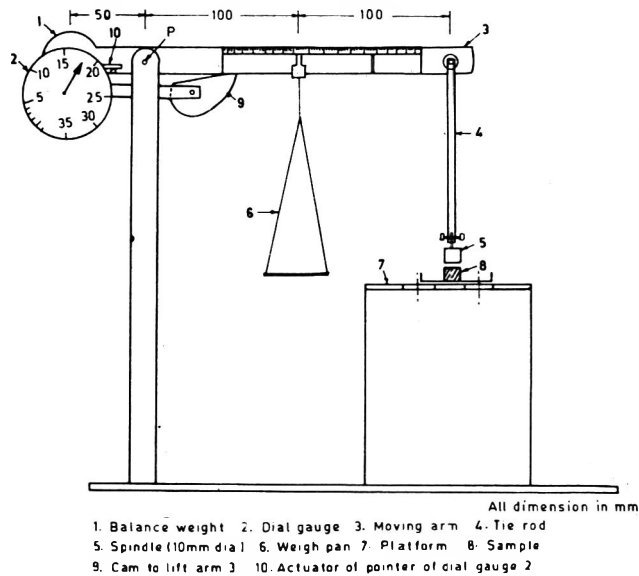


Fig. 2. Texture Measuring Device

dial were calibrated for the movement of the spindle in mm. When a sample of *chhana* was placed just underneath the flat surface of the spindle and its movement was actuated by the cam, the time required for the desired compressive strain on the sample could be noted by a stop watch. By placing appropriate weight on the weigh pan, a constant pressure of 102 g/cm^2 was applied on the sample surface.

Creep test on rasogolla: The above mentioned test was also carried out on *rasogolla* samples (10 mm diameter, 12 mm long). Since cohesiveness and sponginess are the important textural parameters, the *rasogolla* samples were compressed for a second time to the same final height (i.e. 9 mm) after the first compression. The times required for the first (T_{R1}) and the second (T_{R2}) compression were noted. It was considered that for cohesive samples the difference ($T_{R1} - T_{R2}$) between the two compression times would be small. The values of T_{R1} and T_{R2} reported in the paper are the average of three observations.

Temperature rise and expansion of chhana balls: It was assumed that with the change in consistency of *chhana* due to the varying degree of kneading, the expansion and the rise of temperature at the centre of *chhana* balls during cooking would vary. The temperature was measured by placing the bead of a chromel-alumel thermocouple at the centre of the ball and connecting the lead wires from the thermocouple to a digital temperature indicator. During the time of cooking, the diameter of the ball was measured with the help a vernier caliper. The centre temperature and the diameter of the balls were noted at an interval of 0.5 min starting from the time the balls were placed in boiling water.

Sensory evaluation of rasogolla: The quality of the

'market *rasogolla*' and the *rasogolla* made from mechanically kneaded *chhana* were evaluated by a panel of seven trained judges. The quality attributes and the maximum score allotted were: general appearance 10, colour 10, taste 30 and texture 50. The judges were familiarized with the quality of an ideal *rasogolla* as general appearance: spherical, colour: dull white, taste: moderately sweet and texture: smooth, soft and spongy. The evaluation was done with a 10 point hedonic scale to each of the quality attributes.

Results and Discussion

The values of flow behaviour index, n , consistency coefficient, K , modified mixing index, M and time of compression T_C of the mechanically kneaded *chhana* were plotted at four disc speeds in Fig. 3. It is observed from the nature of the curves that the values of n , K , M and T_C have nearly stabilized at 89 cm/sec disc speed. The values of n less than 1 suggests that *chhana* is a pseudoplastic non Newtonian fluid⁷.

The values of n , K and M of the 'market *chhana*' were 0.335, 356 Pa.sⁿ and 15.3 s respectively and those of mechanically ground *chhana* at 89 cm/sec disc speed were 0.345, 368, Pa.sⁿ and 24.8 s representing 2.99, 3.37 and 62.1 percent variation in the respective parameters with respect to the 'market *chhana*'. It may be recalled that the moisture content of the 'market *chhana*' was 60 per cent whereas that of the mechanically kneaded *chhana* ranged between 55 and 58 per cent. The composition of the market *chhana* was not evaluated. A possible difference in composition and the difference in moisture content were probably responsible for the large variation in the time of compression suggesting some textural difference between the 'market *chhana*' and the mechanically kneaded *chhana*.

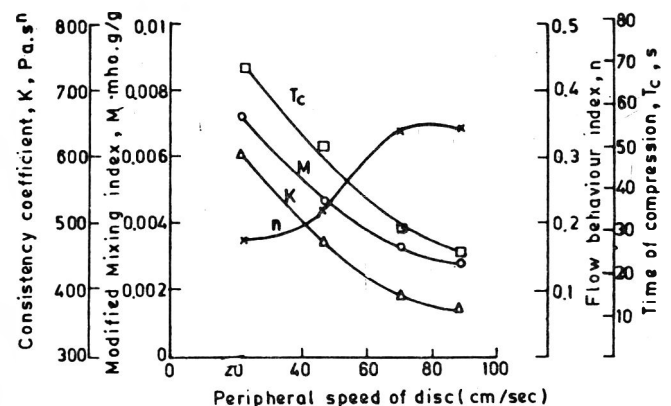


Fig. 3. Flow behaviour index, n , consistency coefficient, K , modified mixing index, M and time of compression, T_C of *chhana* at different peripheral speed of disc.

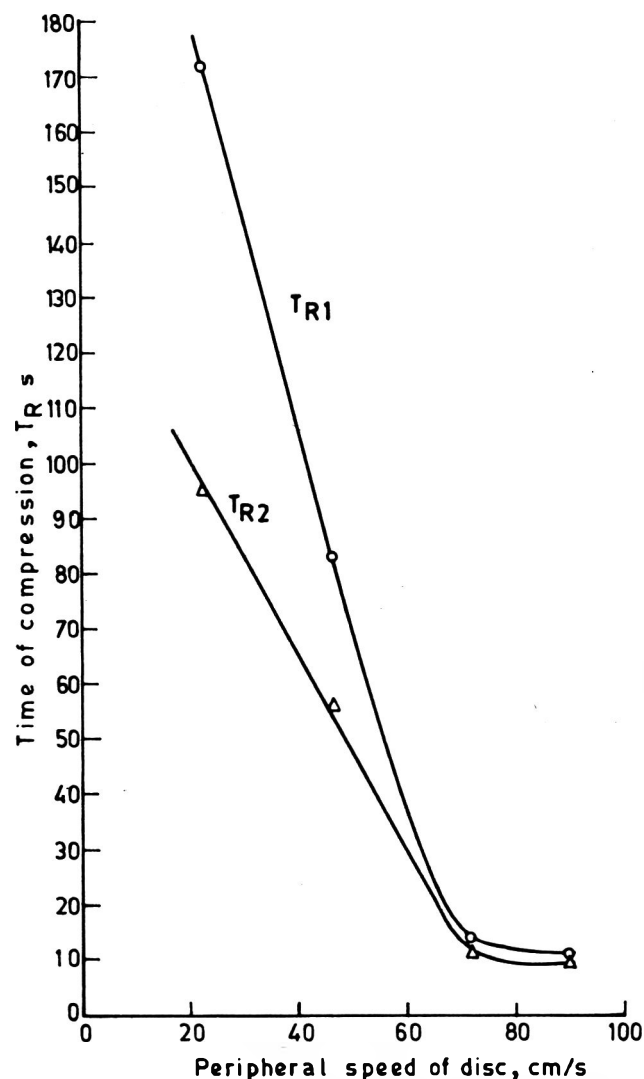


Fig. 4. Time of first compression T_{R1} and time of second compression T_{R2} of rasogolla at different disc speeds.

Fig. 4 shows the relationship between disc speed and the two compression times T_{R1} and T_{R2} for the *rasogolla* made from mechanically kneaded *chhana*. It may be observed from the figure that variation in the values of T_{R1} and T_{R2} is expected to be small beyond 89 cm/sec disc speed. The reduction in the value of $(T_{R1} - T_{R2})$ with the increase in disc speed suggests that *rasogolla* becomes increasingly cohesive and spongy.

At 89 cm/sec disc speed the value of $(T_{R1} - T_{R2})$ for the *rasogolla* was $10.4 - 9.1 = 1.3$ sec. whereas that of 'market *rasogolla*' was $9.2 - 8.3 = 0.9$ Sec. This represents 44.4 per cent variation with respect to the 'market *rasogolla*'. The extent of this difference was probably due to the difference in cooking media (water for mechanically kneaded *chhana* but sugar syrup for market *chhana*) used and the possible experimental error in measuring the times T_{R1} and T_{R2} . A 0.1 sec. difference in the measurement could

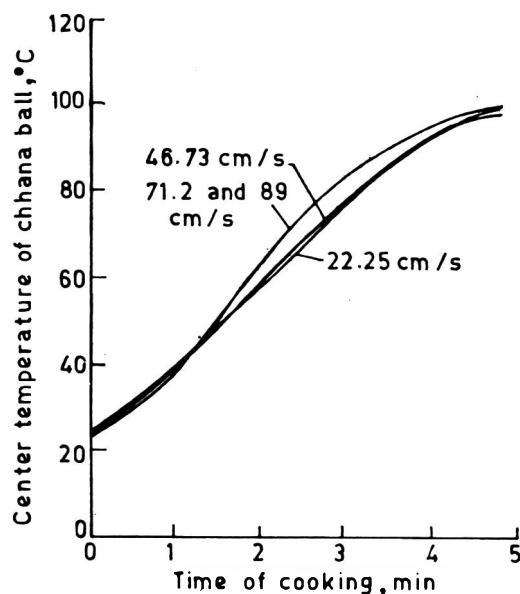


Fig. 5. Centre temperature of chhana ball at various time of cooking and disc speed.

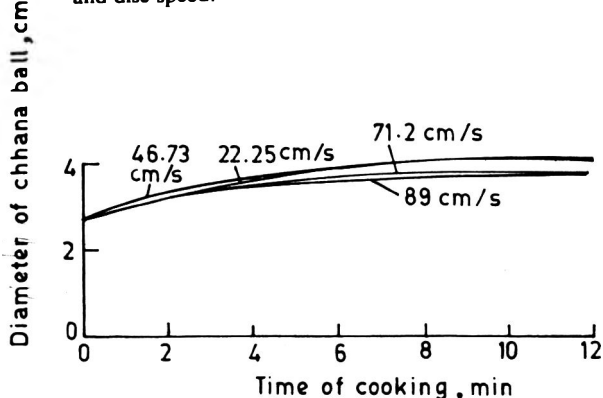


Fig. 6. Diameter of chhana ball at various time of cooking and disc speed.

cause about 10 per cent variation.

Fig 5 represents the variation of centre temperature of *rasogolla* during cooking. It is observed that the centre temperature reaches the maximum within 4.75 to 5 min. The reason for a slightly higher rate of temperature rise with increasing disc speed was probably due to greater homogeneity and lower entrapment of air inside the *chhana* balls.

Fig 6 shows the increase in diameter of *chhana* ball

TABLE 1. SENSORY SCORES OF RASOGOLLA

Disc speed (cm/sec.)	Appearance	Colour	Taste	Texture	Total
22.25	6.71	6.86	24.00	20.71	58.28
46.73	6.71	7.00	23.14	25.71	62.56
71.20	7.43	7.29	24.86	38.57	78.15
89.00	7.57	8.00	24.43	42.86	82.86
M.R.	8.57	8.57	27.43	45.00	89.57

M.R. = Market rasogolla.

after it is placed in boiling water. The initial diameter of the ball was 2.7 cm and it increased to 3.8 to 4.1 cm within 12 min. The reason for lower rate and value of expansion at higher disc speed was probably due to greater cohesiveness and lower stretchability of casein strands.

Table 1 gives the sensory score of *rasogolla*. It is observed from the Table that the quality variation mainly lies with the textural properties of *rasogolla*; not with its appearance, colour and taste. The overall sensory score of 'market *rasogolla*' was 89.57 and that of the *rasogolla* prepared from *chhana* kneaded at 89 cm/sec disc speed was 82.56. The later thus differed by 7.5 per cent from the 'market *rasogolla*'.

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Studies on the Effect of Fattening on Carcass Characteristics and Quality of Meat from Bannur Lambs

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Carcass traits, non-carcass components and some quality characteristics of meat from Bannur ram lambs reared upto 10-11 months of age on range feeding and fattening were compared. Fattened lambs attained 22.7 kg live wt. compared to significantly lower live wt. of 14.5 kg for range fed sheep. The studies indicate high growth potential for Bannur breed when fed under confinement as seen by the much higher dressing yield, meat yield and meat/bone ratio. Also the yield of less valuable parts such as skin, head, blood, etc. as a proportion of live wt. is much lower for fattened sheep. Increased intramuscular fat content due to fattening of lambs did not affect the WHC, cooking loss and thermal shortening of muscles. The markedly lower shear force values for muscles from fattened lambs indicate that fattening produces tenderer muscles than those from range fed ones.

In India, sheep are generally raised by grazing. As the lambs are grazed on deteriorating pastures, they hardly attain the optimal slaughter weight in 10-11 months; the quantitative yield of meat is also poor¹. It has been realised that raising of animals under confinement is becoming inevitable as: (i) the existing grass land is decreasing because of encroachment for agricultural purposes, and (ii) overgrazing has also led to environmental degradation in many regions. Many researchers have studied the effect of feed supplements in basal rations on the fattening of lambs and production of meat²⁻⁶. Relatively little work has been done in India on the influence of feeding under confinement of mutton type sheep breed on meat quality. In the current investigation, Bannur lambs, considered to be the ideal butchers' sheep, were used for studying the influence of fattening of lambs on carcass traits and meat quality.

Materials and Methods

Animals and feeding: Twelve ram lambs of Bannur breed, born at the Govt. Sheep Breeding Farm, Dhāngur, Mandya District, were selected at random based on their weight at birth. Out of this, six lambs were raised at the farm till they attained the age of 10-11 months and the remaining six were brought to the laboratory when they were between the age of 5-5½ months and group fed in the sheep yard by providing concentrate at an average rate of 400 g/sheep/day along with approximately 250 g of fresh green lucerne/grass per sheep per day till 10-11 months. Water was given *ad lib.* The concentrate was prepared by mixing yellow maize (60 per cent w/w), deoiled rice-bran (29 per cent), groundnut cake (10

per cent) and mineral mix containing salt (1 per cent). The feed contained 12 per cent protein on whole weight basis.

Another 12 male yearling sheep born and raised conventionally (range-fed) on the Government Sheep Breeding Farm upto the age of 23-28 months (22-25 kg live weight) were also used for comparative studies.

Slaughter: All the animals were slaughtered in Training Abattoir by Halal method and dressed conventionally as reported earlier⁸; the hot carcass and the various offal parts (non-carcass components) were weighed. The carcasses were chilled at 2-3°C for 24 hr.

Carcass evaluation: The chilled carcasses were weighed and cut into primal cuts, viz., neck, shoulder, rack, loin and leg. The trimmable fat was removed from the cuts and rib-eye area measured. Meat and bone were separated and weighed.

Meat quality studies: The biceps femoris (BF), semi-membranosus (SM) and semi-tendinosus (ST) muscles of both left and right thighs of range fed and fattened lambs were dissected, wrapped in low density polyethylene bags and frozen stored at -15 to -18°C for 1-2 months. The frozen muscle samples of each animal were drawn periodically and thawed in running water. The left thigh muscles were minced separately and the mince was used to determine chemical composition⁹ and water holding capacity (WHC) by the filter paper procedure¹⁰. The right thigh muscles were placed individually in polypropylene pouches, immersed in a water-bath, heated to boil and allowed to remain at this temperature for 20 min. After cooking, the pouches were taken out and the muscles allowed to cool. Each of these was then sampled for

WB shear measurement as described earlier⁸. In each piece, 8-10 readings were taken and the mean value reported for the muscle. The sheared muscle pieces were minced and moisture content and WHC were estimated. The muscle length and weight changes due to cooking were also noted to determine the extent of thermal shortening and cooking loss.

Statistical analysis: The data on muscle composition, thermal shortening, cooking loss, WHC and shear properties of muscles were analysed using Analysis of Variance technique appropriate to the design¹¹ and Duncan's New Multiple Range Test¹² was applied to separate the treatment means.

Results and Discussion

Performance of lambs under feeding regimens: The live weight of lambs at birth, 2.5 ± 0.4 kg (n=12) increased to 12.2 ± 1.5 kg (n=12) under range feeding at the farm upto 5-5½ months. At this stage, 6 lambs from the lot were brought to the laboratory and group fed under confinement. The fattened lambs increased in weight at a faster rate reaching 22.7 kg at the age of 10-11 months compared to range-fed lambs which attained live weight of only 14.5 kg at the same age (Table 1). This is found to correspond to the overall growth rate of 39.3 g/day of age for range-fed as against 68.0 g/day of age for fattened lambs (calculated upto the point of slaughter). This indicates about 60 per cent increase in body weight of fattened lambs over range-fed. Mirajkar² has earlier reported 50-80 per cent increase in body weight of fattened lambs. The carcass yield is also much higher in the case of fattened lambs. It was also noticed that the percentage loss in carcass weight on chilling (2-3°C for 24 hr) is lower in fattened lambs (Table 2) since the presence of higher quantity of fat (back fat and intra-

TABLE 2. NON-CARCASS COMPONENTS (G/Kg BODY WT)

	Range fed	Fattened
Head	76.4 ± 9.6	63.2 ± 4.5
Skin	82.7 ± 18.2	85.2 ± 14.7
Four feet	31.3 ± 8.5	26.0 ± 2.7
Empty stomach	35.9 ± 7.9	26.9 ± 5.2
Lungs	23.7 ± 3.6	20.7 ± 4.8
Liver	24.2 ± 4.8	21.6 ± 5.0
Pancreas	3.2 ± 0.6	2.7 ± 0.7
Heart	3.5 ± 0.6	3.4 ± 0.9
Kidney	3.5 ± 0.9	2.4 ± 0.4
Total separable fat	—	14.3 ± 4.6
Blood	32.4 ± 5.1	27.5 ± 7.6

Values are means ± SD from 6 Bannur crypt rams of 10-11 months age.

and inter-muscular fat) minimises water loss due to evaporation.

Further, it was observed that feeding under confinement only upto the age of 10-11 months could increase the body weight and carcass yield to the optimum level. This is amply evident from the data collected on fattened lambs when compared with yearling sheep (23-28 m) raised by range feeding on the farm. Total meat yield as a proportion of chilled carcass weight was actually lower in the case of yearling sheep (Table 1).

Non-carcass components: It may be observed that yield of less valuable non-carcass components is generally much lower in the case of fattened lambs compared to the range-fed (Table 2).

Carcass components: The improvement in yields of meat in fattened lambs is indicated by the higher meat/bone ratio whereas the proportion of each retail cut to carcass weight does not show much variation in the lambs fed either way (Table 3). The higher rib-eye area (Table 1) and higher meat/bone ratio indicate superior meat producing character of fattened lambs. The data on the yield of retail cuts and meat/bone ratio for fattened lambs are comparable to those of yearling sheep (Table 3). These observations clearly indicate that lambs grow to marketable size in a short period of 10-11 months when fed under confinement.

Muscle composition: All the three thigh muscles (BF, SM and ST) of fattened lambs had considerably lower ($P < 0.05$) water content and higher intramuscular fat (IMF) content than their range-fed counterparts (Table 4). The two parameters are highly negatively correlated ($P < 0.001$), the correlation coefficients being -0.86, -0.83 and -0.94 for BF, SM and ST muscles respectively. However, the variation among the muscles from range-fed or fattened lambs is not significant ($P > 0.05$). This finding that water content varies inversely with IMF content is in line with earlier observations of Hamm¹³, Lawrie¹⁴ and

TABLE 1. LIVE WEIGHT AND CARCASS YIELDS

	Male lambs*		Male yearlings**
	Range fed	Fattened	
Live wt., (kg)	14.5 ± 2.7	22.7 ± 1.1	23.3 ± 4.1
Carcass wt. (hot) (kg)	6.2 ± 1.2	10.7 ± 1.1	11.4 ± 2.4
% Dressing (hot)	42.3 ± 1.2	48.3 ± 4.0	48.6 ± 4.2
% Carcass wt loss on chilling	5.2 ± 1.0	4.4 ± 1.9	3.8 ± 1.3
Total meat as % chilled carcass wt.	65.8 ± 3.7	68.5 ± 2.5	59.1 ± 3.5
Total bones as % chilled carcass wt.	30.8 ± 4.1	25.1 ± 1.9	—
Separable fat as % chilled carcass wt.	—	3.1 ± 0.7	—
Rib-eye area (cm ²)	11.3 ± 1.6	14.4 ± 2.1	11.4 ± 0.8

*10-11 months, n=6 **23-28 months, n=12

Values are means ± SD

TABLE 3. YIELD AND MEAT/BONE RATIO OF RETAIL CUTS

	Male lambs					
	Range-fed (n=6)		Fattened (n=6)		Male yearlings (n=12)	
	% yield*	M/B**	% yield*	M/B**	% yield*	M/B**
Neck	8.1 ± 0.6	1.9 ± 0.3	6.7 ± 1.3	2.1 ± 0.3	6.1 ± 1.6	1.5 ± 0.3
Shoulder	29.1 ± 2.2	1.9 ± 0.3	27.6 ± 0.8	2.6 ± 0.8	30.0 ± 2.5	3.2 ± 0.5
Rack	13.9 ± 1.0	1.7 ± 0.3	15.3 ± 1.4	2.2 ± 0.3	15.7 ± 1.2	2.5 ± 0.6
Loin	9.2 ± 1.4	2.7 ± 0.6	9.6 ± 2.0	4.1 ± 1.0	10.7 ± 1.4	3.7 ± 0.6
Leg	36.8 ± 1.5	3.0 ± 0.3	35.5 ± 2.0	3.6 ± 1.7	23.9 ± 2.3	3.5 ± 0.9
Total retail cuts	—	2.2 ± 0.4	—	2.8 ± 0.4	—	3.0 ± 0.4

Values are means ± SD, *Yield expressed as % chilled carcass wt.; **M/B: Meat/Bone ratio. Male lambs are of 10-11 months and male yearlings are 23-28 months.

TABLE 4. COMPOSITION OF UNCOOKED MUSCLES

Treatment groups		Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Moisture/protein ratio
Range-fed	BF	77.61a	1.18a	19.81a	1.10a	3.92a
	SM	77.41a	1.19a	19.30a	1.12a	4.03a
	ST	77.74a	1.01a	18.87a	1.06a	4.12a
Fattened	BF	74.72b	2.66b	19.58a	1.11a	3.82a
	SM	75.05b	3.03b	18.82a	1.14a	4.00a
	ST	75.32b	3.04b	18.77a	1.11a	3.98a
SEm (30 df)		±0.33	±0.14	±0.36	±0.04	±0.04

Values are means ± SD from 6 Bannur crypt rams 10-11 months old, muscles stored frozen for 1-2 months.

Means in the same column with different superscripts differ significantly ($p < 0.05$) according to Duncan's New Multiple Range Test.

Asghar and Pearson¹⁵.

The total protein and ash contents of muscles are not affected by the method of rearing of lambs. Summers *et al*¹⁶, have shown that lambs fed on pasture alone yielded carcasses that had more protein, moisture and ash with less fat. Sawyer¹⁷ noticed that the ash content in different cuts of meat of lamb, beef and pork is around 1 per cent and that it is unaffected by variability in moisture and fat contents.

Thermal contraction, WHC and tenderness of muscles: The fattened lambs have yielded larger

($P < 0.001$) muscles than range-fed ones and when cooked, these muscles suffer thermal contraction to different degrees depending upon the type of muscle and not on the method of rearing. Similarly, WHC of cooked as well as un-cooked muscles and cooking loss were not significantly ($P > 0.05$) different between muscles of range-fed and fattened lambs. As regards moisture content of cooked muscles, the values are significantly ($P < 0.05$) lower for fattened lambs than for range-fed ones; this was also true of uncooked muscles. Miller *et al*¹⁸, reported that WHC decreased

TABLE 5. WHC, COOKING LOSS AND WB SHEAR VALUES

Treatment groups	Length of muscles (cm)	% shortening in length on cooking	Water holding capacity (%)			% moisture in cooking meat	% cooking loss	WB shear values
			Un-cooked muscles	Cooked muscles				
Range-fed	BF	14.58a	38.66a	59.10a	62.12a	67.71a	30.96a	10.91a
	SM	9.33b	20.08b	55.87a	54.71b	66.88a	36.25a	12.94a
	ST	11.52c	32.40c	57.63a	54.94b	68.38a	30.58a	8.04b
Fattened	BF	19.27d	37.92a	59.30a	62.49a	63.82b	31.32a	4.60c
	SM	11.35c	22.02b	58.66a	53.42b	63.32b	33.15a	7.44b
	ST	13.17ac	30.63c	59.31a	55.93b	64.45b	31.82a	6.33bc
SEm (30df)		±0.60	±1.67	±1.83	±2.06	±0.66	±1.41	±0.72

Values are means ± SD from 6 Bannur crypt rams, 10-11 months old. Carcass held under Achilles tendon suspension at 2°C for 24 hr. Muscles stored under frozen conditions for 1-2 months.

Means in the same column followed by different superscripts differ significantly ($P < 0.05$) according to Duncan's New Multiple Range Test.

as the fat content of meat was increased which they attributed to the increase in the moisture/protein ratio in the beef muscles. However, in the present study the moisture/protein ratios (Table 4) were in the range of 3.82-4.12 for thigh muscles (BF, SM, ST) and the variation due to feeding regimen was not significant ($P>0.05$).

In respect of tenderness, the data on WB shear values (Table 5) of three thigh muscles from both range-fed and fattened lambs indicate that the muscles from fattened lambs have lower shear values than those from range-fed lambs, the decrease being significant ($P<0.05$) for BF and SM and marginal ($P>0.05$) for ST muscles. These findings indicate that higher degree of marbling provides tenderer muscles possibly due to insulating effect of fat as also reported by other researchers in beef¹⁹, pork²⁰ as well as lamb^{21,22} muscles.

In conclusion, the feeding of Bannur lambs under confinement (fattening) substantially improved both yields of carcass/meat and tenderness of muscles. The improvement in tenderness was related to marbling effect brought about by fattening of lambs.

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Studies on Differentiation of Cattle Meat From the Meat of Other Species of Animals. II. Comparative Efficacy of Different Serological Methods

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Efficacy of different serological tests like DID, CIE, IE and micropassive haemagglutination (HA) was studied in detecting cattle meat from the meat of other species of animals like buffalo, sheep, goat, pig, chicken and donkey by using RACM and BACM sera. The HA test was observed to cause nonspecific haemagglutination, while IE though observed to be specific was least sensitive. Both DID and CIE were equally specific and sensitive. However, DID test was most suitable from the point of view of simplicity and reliability. Examination of different factors like concentration of agarose, buffers/NSS used to suspend agarose and pH of buffers used showed that 0.6% agarose in NSS or in PBS pH 7.5 gave best results. Where facilities are available, CIE test using 0.6% agarose in Williams and Chase buffer with constant current of 5 mA/75×25 mm slide for 30 min would be the choice.

Cattle meat cannot be sold because cattle slaughter is not permitted by law in most parts of India. It is therefore, sold clandestinely not as cattle meat but in the name of meat of other species of animals like buffalo, sheep and goat or by adulterating these meats. A method which would be simple and reliable to detect such adulteration would, thus, be of great help. The present studies, therefore, were undertaken to investigate the comparative specificity and sensitivity of the different serological tests like double immuno diffusion (DID), immuno electrophoresis (IE), counter immuno electrophoresis (CIE) and micro passive haemagglutination (HA) to differentiate and identify cattle meat from other kinds of meat.

Materials and Methods

Antigens and antibodies: The freeze-dried skeletal muscle extract antigens of cattle (CFD), buffalo (BFD), goat (GFD), sheep (SFD) and the field antigens derived from muscle or organ extracts of different species of animals like cattle, buffalo, sheep, goat, chicken, pig and donkey were used. The monospecific rabbit-anti-cattle serum (RACM) and concentrated monospecific buffalo-anti-cattle serum (BACM) were used as the antibody sources. The antigens and antibodies were prepared as described by Bansal and Mandokhot¹.

Serological tests: The various serological tests used to differentiate cattle meat from that of other species of animals were DID², IE^{3,4} and HA^{5,6}. The effect of different variables like temperature (20, 25, 37 and 40°C) and duration of incubation (4 to 72 hr), diluents

used for agarose gel (PBS pH 7.3 and NSS for DID test and veronal buffers^{7,8} for CIE and IE tests) and agarose concentration (0.5, 0.6, 0.7, 0.8, 0.9%) on these tests was also studied. Besides the above mentioned variable for IE and CIE tests, the effect of amount of constant current supply (10, 15, 20 mA/ 75 × 25 mm slide), duration (90 to 105 min for IE and 20 to 35 min for CIE) of electrophoretic run was also studied. The antigens used were CFD, BFD, SFD, GFD in 50, 25, 12.25, 6.125 and 3.6 mg/ml concentrations. The antibody used was undiluted BACM serum.

Specificity of the test: The undiluted BACM and RACM sera were allowed to react with CFD, BFD, SFD, GFD antigens as well as with the homologous and heterologous field antigens. In all, 260 field antigens were tested by DID and CIE tests of which 146 were from cattle (Muscle 42, liver, kidney, spleen, heart and lungs 18 each, blood serum 10, adrenal glands 4). The rest of the antigens (114) were derived from the muscles and organ extracts of heterologous species like buffalo (Muscles 43, kidney and lungs 5 each, liver, spleen 4 each and heart 2) and muscle extract from sheep (22), goat (18), chicken (5) pig (5) and donkey (2). But for the IE test, only 17 field antigens comprising 5 muscle extract antigens of cattle (CME) and two each of the muscle extract of buffalo (BME), sheep (SME), goat (GME), pig (PME), chicken (CHME) and donkey (DME) were used for differentiation.

Sensitivity of the tests: The sensitivity of the DID, IE and CIE tests was studied by using two fold

dilutions (upto 1:128) of the BACM and RACM sera and 50 mg to 0.1952 mg/ml of the CFD antigen. The protein concentration in the CFD and CME antigens was estimated by the method of Lowry *et al*⁹.

HA test: Freeze-dried antigens of different species (cattle, buffalo, goat and sheep) were first dissolved in phosphate buffer solution (0.01 MPBS), pH 7.2@ 50,25, 12.5, 6.25, 3.125, 1.562, 0.781, 0.3905, 0.1952, 0.0976 and 0.0488 mg/ml and filtered through Whatman filter paper No. 4 before use.

Preparation of sheep red blood cells (SRBC): Sheep blood was collected in equal volume of modified Alservar's solution (dextrose 20.5 g, trisodium citrate 8.02g, citric acid 0.55 g, sodium chloride 4.2 g and dist water 1000 ml) and kept in a refrigerator for 3 days. The erythrocytes were separated and washed thrice with PBS, pH 7.2, packed by centrifuging at $600 \times G$ for 10 min and resuspended in PBS, pH 7.2 to obtain 2.5 percent suspension.

Tanning of SRBC: Equal volumes of 2.5 per cent SRBC and freshly prepared tannic acid (1:20000) were mixed and incubated at 37°C in a water bath for 15 min. After incubation, the mixture was centrifuged at $600 \times G$ for 10 min and the supernatant discarded. The tanned SRBC were washed once with equal volume of PBS, pH 7.2 to bring it to the original concentration. The suspension was stored at 4°C and used within 24 hr.

Preparation of diluent: Non-immune rabbit serum inactivated at 56°C in a water bath for 30 min was absorbed with tanned SRBC (2 ml of serum + 2 ml of 2.5 per cent tanned SRBC at 37°C for 15 min) to prevent non specific agglutination and centrifuged at $600 \times G$ for 10 min. The clear supernatant serum was diluted to 1:100 with PBS, pH 7.2 and was used as a diluent for BAC or RAC monospecific sera.

Sensitization of SRBC: Four volumes of PBS (pH 6.4) one volume of antigen (Freeze-dried or muscle extract of sheep/cattle/buffalo/goat) and one volume of tanned SRBC were mixed in the said order. The mixture was incubated for 15 min at 37°C, centrifuged $600 \times G$ for 10 min, Washed with two volumes of serum diluent and resuspended in one volume of serum diluent. The sensitized SRBC were stored at 4°C and used within 48 hr.

The test proper: The SRBC sensitized with cattle, sheep, goat and buffalo antigens (0.5 ml) were transferred separately to the wells of microplates to which 0.05 ml of BACM or RACM serum was added and mixed gently by shaking the plate. Simultaneously, various controls for tanned SRBC, sensitized SRBC and for non-specific agglutination were also maintained by mixing 0.05 ml of serum diluent with 0.05 ml of

tanned SRBC, with 0.05 ml sensitized SRBC and 0.05 ml of tanned SRBC with 0.05 ml of BACM/RACM serum, respectively. The microplates were incubated at 4°C for 4 hr and the results noted. The presence of agglutination in the wells containing SRBC sensitized with cattle FD antigen but no agglutination in all other wells including the controls was considered to be a positive reaction.

Detection of adulteration of meat: The DID and the CIE tests were used to detect adulterant cattle meat in buffalo meat, mutton and chevon. The procedure followed for the detection was the same as described by Bansal and Mandokhot.¹

Results and Discussion

It had been observed that about 25 to 30 per cent of meat sold in various parts of our country is adulterated with other kinds of meat¹⁰. Thus, a method which would be both specific and sensitive enough to detect the adulteration at minimum possible level would be of great help. Hardly any work has been carried out on this aspect in our country and the reports are inadequate and contradictory.^{8,11-15} Differences in esterase enzyme pattern had been used to differentiate a pure beef sample from that of a pure buffalo meat sample¹¹. How far this method would be applicable to identify adulterated meat such as cattle meat in buffalo meat is, however, doubtful. The use of IE test using unabsorbed diagnostic sera raised in rabbits to differentiate meats of buffalo, cattle, sheep and goat was also reported⁸. The differentiation was made on the basis of number and position of precipitation arcs formed. Surprisingly, however, no precipitation reaction between the cattle and buffalo meat using unabsorbed RAB serum (anti-buffalo rabbit serum) or sheep and goat meat using unabsorbed RAC serum was observed. The DID test was used by Tagore *et al*¹², Pandey and Pathak¹³, Sherikar *et al*¹⁴, and Reddy¹⁵. All of them except Tagore *et al*¹², who did not give details of the antigen or antibody used, failed to identify the meat of cattle from buffalo using the diagnostic sera developed in the rabbits. The present comparative study of the DID, IE and the HA tests was, therefore, undertaken to find out their specificity sensitivity, rapidity and simplicity to identify and differentiate cattle meat from that of other species of animals employing RACM as well as BACM sera. The CIE test, though not tried earlier anywhere for this purpose, was also included in the study as it is otherwise known to be a rapid serological test.

The HA test was observed to cause non-specific haemagglutination indicating the necessity of more purified antigen and antibody system for this particular test. Besides, the test was also found to be

cumbersome, time consuming and comparatively difficult to perform. The other three tests were found to be equally specific as all of them revealed a positive precipitation reaction between the RACM/BACM serum and the homologous antigen (CFD/CME) but not with the heterologous (BFD/BME, GFD/GME, SFD/SME, CHME, PME and DME) antigens. The specificity of the DID, IE and CIE tests was further confirmed as the results were found to be reproducible with field antigens examined. None (114 for DID and CIE tests and two each of BME, GME, SME, PME, DME & CHME for the IE test) of the heterologous field antigens tested, produced visible precipitation reaction with the RACM and the BACM sera, but all the homologous antigens (146 tested by DID and CIE and 5 by IE test) formed the line of identity with the known homologous CFD/CME antigen.

As far as the sensitivity of the tests was concerned, both the DID and the CIE tests were equally good. The amount of cattle antigen that could be detected by the DID and CIE tests using different dilutions of the RACM or the BACM serum was observed to be the

same (Table 1). The minimum cattle protein that these two tests could detect by using undiluted BACM or RACM serum was 176.8 µg cattle protein/ml present in 1.562 mg/ml of CFD antigen (Table 1). Parallel sensitivity of both the tests was further proved when both the tests were found to detect 396 (2.5 per cent adulteration) and 198 (1.25 per cent adulteration) µg cattle protein/ml of adulterated buffalo meat by employing BACM and RACM sera respectively. The minimum level of adulterant cattle protein in the mutton and the chevon detected by both the tests was 396 µg/ml (2.5 per cent adulteration) irrespective of the sera used. In comparison with these two tests, the IE test was observed to be much less sensitive, as the minimum amount of CFD antigen that it could detect was limited to 12.5 mg/ml containing 1415 µg cattle protein/ml (Table 1).

As far as the rapidity of the test was concerned, the CIE test was found to have an edge over the DID test as the visible precipitation reaction, in general, appeared 6 to 12 hr earlier in the CIE test. The IE test was the slowest to reveal positive precipitation reaction.

Examination of the different factors like concentration of agarose, use of different buffers/NSS and its pH, revealed that 0.6 per cent agarose in NSS or PBS, pH 7.3 gave equally good results. The DID test was thus found most suitable from the point of view of simplicity also. The CIE test using 0.6 per cent agarose in Williams and Chase buffer⁷ with constant current of 5 mA/75×25 mm slide for 30 min, however, would be the first choice where facilities are available (immuno electrophoresis set and steady supply of electricity).

On the basis of the results obtained, it is apparent that the DID test using 0.6 per cent agarose gel in NSS is the choice test where laboratory facilities are elementary and serum supply is limited. Otherwise, the CIE test would be the test of choice.

TABLE 1. SENSITIVITY OF CIE, DID AND IE TESTS IN DETECTING CATTLE ANTIGEN (CFD) USING RACM AND BACM SERA

Serum dilution	Conc. of CFD (mg/ml)						
	50	25	12.5	6.25	3.125	1.562	781
	Conc. of protein (µg/ml)						
	5660.0	2830.0	1415.0	707.5	353.7	176.8	88.4
Undiluted	D+	D+	D+	D+	D+	D+	D-
	C+	C+	C+	C+	C+	C+	C-
	I+	I+	I+	I+@	I-		
1/2	D+	D+	D+	D+	D-		
	C+	C+	C+	C+	C-		
	I+	I+	I-	I-			
1/4	D+	D+	D+	D-			
	C+	C+	C+	C-			
	I-	I-	I-				
1/8	D+	D+	D-				
	C+	C+	C-				
	I-	I-					
1/16	D+	D-					
	C+	C-					
1/32	D+	D-					
	C+	C-					
1/64	D-	C-					

D = DID test; C = CIE test; I = IE test

+ = Precipitation between RACM or BACM serum and CFD antigen

- = No precipitation between RACM or BACM serum and CFD antigen

0.05 ml of different dilutions of both antigen and antibody used

@ Precipitation reaction between BACM serum and CFD antigen but not between RACM & CFD antigen.

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Studies on Canned Strained Baby Foods Based on Vegetables. II. Green Peas

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Canned strained baby food based on green peas was developed. Thermal process time of 61 min was found necessary to achieve F_0 value of 4 at 115.5°C. Cut-out analysis of the canned product indicated that the product was safe microbiologically. The product was found to be a good source of protein (30% on dry wt basis) and minerals and vitamins. Thermal processing caused a decrease in ascorbic acid (37%), thiamine (82%), and of essential amino acids like lysine (10%), isoleucine (19%), methionine (13%) and threonine (19%). However, the canned strained green peas based baby food showed growth promoting efficiency almost similar to that of commercial infant milk food in young weanling rats.

There is a great demand for the development and manufacture of infant and baby foods by the food processing industry. In India, infant foods based on cereals and milk have gained commercial viability and also consumer demand. Surprisingly, baby foods based on fruits and vegetables are yet to be manufactured and marketed in India. However, the technology of preparing strained baby foods based on fruit and vegetable purees seem to be basically similar to that of canned foods. Processing details and various combinations of single and mixed fruit and vegetable purees for the production of baby foods have been described by earlier workers. Studies on the proximate composition of baby foods indicated them to be good sources of protein, carbohydrates, minerals and vitamins¹⁻⁵. Thermal processing has been found to affect chlorophyll and certain vitamins.⁶⁻⁸ The nutritive value of baby foods evaluated by rat bio-assay methods was found to be satisfactory^{2,9-10}.

Green peas, a good source of protein and minerals, may make a nourishing food for babies. Investigations were, therefore, taken up to (a) standardize conditions to prepare puree from green peas (*Pisum sativum*) with minimal colour and nutrient loss; (b) evolve a thermal process schedule to can strained green peas puree; (c) study the nutritional profile of canned strained green peas baby food; and (d) evaluate its nutritional quality by rat bio-assay.

Materials and Methods

Tender and succulent green peas of optimum maturity were procured from the local horticultural society and used in all the experiments.

i) *Blanching in water*: Shelled green peas were blanched in water at 68° and 82°C. for 5,10,15,20 and 25

min; at 93°C for 4, 8 and 12 min; and at 100°C for 4 min. Blanched peas were cooled in running water and blended in a Stephan Universal machine with a blender attachment. The puree was strained by passing through a 60 mesh stainless steel sieve, filled hot (85°C) into cans of 8 oz capacity (301 × 206 size) and processed at 115.5°C

ii) *Blanching in salt solutions*: Shelled green peas were blanched at both 68°C for 4 min and at boiling temperature for 4 min in solution I, containing magnesium oxide (0.2%) and sodium bicarbonate (0.1 per cent) and solution II containing sodium citrate (1.5%) and processed at 115.5°C.

iii) *Pre-soaking and blanching*: Shelled green peas were first soaked in 2 per cent sodium carbonate solution for 1 hr at ambient temperature (25° ± 2°C) and then blanched in boiling solution of magnesium oxide (0.2 per cent) and sodium bicarbonate (0.1 per cent) for 4 min and processed as described under (i) above.

iv) *Addition of magnesium oxide during pulping*: Magnesium oxide at 0.040, 0.081, 0.121, 0.162 and 0.242 per cent concentrations was added to the presoaked and blanched green peas as in (iii), while pulping was in progress and processed at 115.5°C.

Canning: Plain, AR lacquered and SR lacquered cans were tried for their suitability.

Heat penetration studies: Heat penetration rate into SR lacquered cans of 8 oz capacity (301 × 206 size) filled with hot strained green peas puree (85°C), was measured using Ecklund non-projecting plug-in needle type thermo couples. The temperature during heating and cooling was recorded every two. min. Triplicate runs, consisting of four cans for each run,

were carried out.

Process time calculations: Process time for strained green pea puree was evolved by the general method to achieve the classical F_0 values of 3 to 5 recommended for baby foods¹¹⁻¹³. The values thus obtained were cross-checked with those obtained by the formula method using the formula of Ball¹⁴.

Inoculated pack studies: The validity of the process time calculated to achieve commercial sterility was further tested by inoculated pack studies as described in the NCA manual¹¹.

Physical methods: The reflectance colour, was measured between 415 and 685 nm at an interval of 30 min in a Bausch and Lomb spectronic 20 spectrophotometer with reflectance attachment; values were calculated by the weighted ordinate method¹³. Gross weight of the canned product was obtained by weighing the cans before opening. Particle size for the pulps was determined by passing a known amount of puree over a standard sieve (250 μ mesh) for a known time. Weight of the sieved puree was used to calculate per cent retention of puree on the sieve.

Analytical methods: Moisture, titratable acidity, total ash, crude fibre, ether extractives, protein ($N \times 6.25$, Micro-kjeldahl method) and starch by acid hydrolysis were estimated by AOAC methods¹⁵. Reducing and total sugars were estimated according to the modified Somogyi method¹⁶.

Calcium, iron, magnesium, manganese sodium, potassium, copper, zinc and lead were analysed by atomic absorption spectrophotometry using Instrumentation Laboratory aa/ae spectrophotometer (751 model). Phosphorus and tin estimations were carried out following standard methods^{13,17}. Total carotene, β -carotene, ascorbic acid and thiamine were analysed as described under AVC methods¹⁸.

The amino acid composition was determined in a LKB α -amino acid analyser equipped with a programmer and integrator. Sample for analysis was prepared according to the procedure of Moore and Stein¹⁹.

Chlorophyll was estimated by the Comar method²⁷. Non-enzymatic browning as colour absorbance of alcoholic extracts treated with benzene was measured at 420 nm¹³.

Microbial counts: Total bacterial, *E. coli* and yeast and mould counts were done following standard methods^{15,21}.

Studies on supplementary value: Supplementary value of canned strained green peas based baby food was determined using 28-day old weanling rats weighing 35 to 40g. They were distributed into two groups of 10 each in a completely randomized block design equally according to sex and body weight and

housed individually in cages. The control diet at 10 per cent protein level, consisted of commercial infant milk food (45.5g), corn starch (44.5g) and sugar (10g), cooked with 5.5 times water to gelatinize the starch. The experimental diet contained canned strained green peas based baby food at 20 per cent supplementation level on dry weight basis to the milk diet. Rats were fed *ad libitum* with food and water for a period of 8 weeks. Daily food consumption records and weekly weight records were maintained. At the end of the experimental period, rats were sacrificed and estimations of haemoglobin content and RBC count were done from the blood drawn from an incision in the heart^{22,23}. Livers were wiped free of blood and weighed and examined. The data were analysed statistically by students 't' test.

Results and Discussion

Preparation of strained green peas puree: Thermal processing has been found to affect the pigments, specially chlorophyll. Degradation of chlorophyll was found to be high at low pH and prolonged processing. Soaking shelled peas in the soak solution (2 per cent sodium carbonate) for 1 hr followed by blanching in boiling solution containing magnesium oxide (0.2 per cent) and sodium bicarbonate (0.1 per cent) for 4 min resulted in 62 per cent retention of green colour (Table 1). Addition of magnesium oxide (0.161 per cent), while pulping the presoaked and blanched peas improved chlorophyll retention (82 per cent) with a pH of 8.82. Chlorophyll retention was found to depend upon the type of can used for processing,

TABLE 1. EFFECT OF PRE-TREATMENTS ON VISUAL COLOUR, pH, CHLOROPHYLL RETENTION AND REFLECTANCE COLOUR OF GREEN PEAS PUREE

Pre-treatments	Visual colour	pH	Chlorophyll retention (%)	Reflectance colour			
				x	y	Y%	D (nm)
I*	Slightly greenish yellow	6.97	61.0	0.3350	0.3783	40.5	567.1
II**	Greenish yellow	8.82	82.0	0.3347	0.3904	39.4	565.0
III***	Olive green	8.23	88.0	0.3331	0.3666	35.9	565.4

* Presoaking in sodium carbonate solution (2%) for 1 hr and blanching in boiling solution containing magnesium oxide (2.2%) and sodium bicarbonate (0.1%) for 4 min.

** Addition of magnesium oxide (0.161%) to presoaked and blanched green peas as in I.

*** Processing of green peas puree given treatments as in I and II in SR lacquered cans.

being better in sulphur resistant lacquered (SR lacquered) cans. These results indicate that the colour of green peas could be retained maximum at a pH of 8.3.

Heat penetration studies: The heat penetration curve for the cans filled hot at 85°C with strained green pea puree exhibited conduction type of heating.

Process time calculated by the graphical and formula method: Strained green pea puree processed in 301 × 206 size cans required a process time of 61 min to reach a sterility value of 4 which was recommended for such baby foods^{12,13}. The process times calculated were close to those obtained by the graphical method. The calculated process times were valid as checked by inoculated pack studies and were sufficient.

The canned strained green peas puree will be referred to as canned strained green peas baby food (PBF).

Product profile of PBF at various stages of processing: The product profile of PBF indicated that it was greenish yellow, smooth and flowy with characteristic pea aroma and taste. The suggested pre-treatment raised the pH of the puree to 8.7. However, heat processing reduced the pH of the product to 7.9 but the visual colour was still greenish yellow.

The reflectance colour data (Table 2) indicated a definite colour difference between fresh peas and PBF. During processing, a decrease in brightness (Y per cent) was observed followed by a shift in the dominant wavelength (D) from greenish yellow to yellow region in the chromaticity diagram. This indicated that processing affected the green colour of peas to a considerable extent.

The strained green pea puree retained 95 per cent of chlorophyll while the PBF retained 90 per cent of that of fresh peas. The loss of the pigment in the PBF can be attributed to decrease in pH from 8.7 to 7.9. Similar observations were reported by earlier

TABLE 2. REFLECTANCE COLOUR DATA, CHLOROPHYLL RETENTION AND ABSORBANCE OF PBF.

Particulars	Reflectance				Chlorophyll retention (%)	Absorbance at 420 nm
	x	y	Y%	D(nm)		
Green peas fresh	0.3395	0.3587	42.39	572.4	-	-
Strained green peas puree	0.3451	0.3692	38.81	573.6	94.49	0.236
PBF	0.3347	0.3530	31.05	576.6	89.98	0.249

Chlorophyll in fresh peas 37.79 mg/100 g dry matter

TABLE 3. EFFECT OF PROCESSING ON THE PROXIMATE COMPOSITION OF PBF

Constituents ^a (g/100 g DM)	Green peas		
	Fresh	Strained puree	PBF
Protein (N × 6.25)	30.73	30.54	29.69
Ether extractives	1.02	1.00	0.99
Total ash	3.31	3.86	3.88
Ash soluble in HCl	3.02	3.31	3.31
Acidity*	0.23	0.15	0.17
Sugars—Total	4.65	4.73	4.76
Reducing	0.35	0.32	0.33
Starch	18.52	18.64	18.92
Crude fibre	8.90	4.69	4.67
Other carbohydrates (by diff)	32.63	36.36	36.92

a: Each value is the mean of triplicate analysis

*as anhydrous citric acid.

workers^{6,24}. Canning of strained green peas puree did not bring about non-enzymatic browning.

Microbial counts: The total count, *E.coli* and yeast and mould counts of PBF were found to be nil after 72 hr incubation. Thus, the PBF was found to be safe and conformed to the ISI specifications for infant milk food²⁵.

Nutrient composition of PBF at various stages of processing: The proximate composition and mineral and vitamin contents of PBF are given in Tables 3 and 4. Processing affected certain nutrients. However, proximate constituents did not change during processing. Mineral constituents were quite stable. Tin pick-up was also minimum (7 per cent) during

TABLE 4. MINERAL AND VITAMIN CONTENTS OF PBF Green peas

Constituents ^a	Green peas		
	Fresh	Strained puree	PBF
Calcium(mg/100g)	186.6	172.1	169.4
Phosphorus(mg/100g)	491.0	486.7	489.0
Iron(mg/100g)	36.4	35.5	33.6
Magnesium(mg/100g)	133.1	262.6	258.9
Manganese(mg/100g)	0.9	1.0	0.9
Sodium(mg/100g)	97.1	286.8	248.9
Potassium(mg/100g)	987.6	914.7	905.1
Copper(mg/100g)	0.9	0.8	0.8
Lead(mg/100g)	0.6	0.6	0.4
Zinc(mg/100g)	4.4	4.3	4.5
Tin(mg/100g)	14.8	14.8	15.8
β-carotene(mg/100g)	0.4	0.3	0.3
Ascorbic acid(mg/100g)	32.4	27.9	20.3
Thiamine (μg/100g)	114.8	96.0	20.8

a: Each value is mean of triplicate analysis values are on dry matter basis

processing as the product belonged to low-acid category. Loss of ascorbic acid (37 per cent) and thiamine (82 per cent) were observed in the canned product. The extent of decrease in amino acids during processing is threonine, 19 per cent; isoleucine 19 per cent; alanine 13 per cent; methionine 13 per cent and lysine 10 per cent (Table 5).

Studies on supplementary value: Data on the effect of supplementing PBF at 20 per cent level to milk diet are presented in Table 6. The growth of animals on the experimental diet was closely similar to that of the controls. Liver weight, haemoglobin content and RBC count of both control and experimental groups were very close and did not differ significantly.

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TABLE 5. AMINO ACID COMPOSITION (G/100 G PROTEIN) OF FRESH GREEN PEAS AND PBF

Amino acid	Green peas fresh	PBF
Alanine	4.21	3.65
Arginine	6.40	6.40
Aspartic acid	9.22	5.79
Glutamic acid	15.62	15.82
Glycine	3.73	3.70
Histidine	1.86	2.00
Isoleucine	2.09	1.72
Leucine	5.68	5.66
Lysine	6.14	5.51
Methionine	0.54	0.47
Phenylalanine	3.51	3.42
Proline	3.12	3.62
Serine	4.64	4.45
Threonine	2.70	2.19
Tyrosine	2.49	1.88
Valine	2.09	1.99

TABLE 6. EFFECT OF SUPPLEMENTING MILK DIET WITH PBF AT 20% LEVEL ON GAIN IN BODY WEIGHT, FOOD INTAKE, HAEMOGLOBIN CONTENT, RBC COUNT AND LIVER WEIGHTS OF WEANLING RATS

	Initial wt. (g)	Daily food intake (g)	Gain in body wt.		Haemoglobin (g/dl)	RBC count (mil/dl)	Liver wt (g)
			4 wk.	8 wk.			
Control group	39.80	12.50	69.50	138.80	15.42	7.37	6.90
Exp. group	39.80	10.90	72.00 ^{NS}	137.10 ^{NS}	15.90 ^{NS}	7.23 ^{NS}	7.10 ^{NS}
SE _m			±0.42	±0.10	±0.77	±1.34	±0.27

S.E_m: Standard error of means (18 df)

N.S.: Not significant.

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Storage Studies on Canned Strained Baby Foods Based on Carrot and Green Peas

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Canned strained carrot baby food (CBF) and canned strained green pea baby food (PBF) were evaluated for certain physico-chemical and sensory parameters during storage for 180 days at normal (27°C and 65% RH) and accelerated (38°C and 92% RH) conditions. Both carrot and green pea baby foods kept well for 180 days at normal storage conditions, but they could be stored without much change upto 135 days under accelerated conditions. Changes in moisture, acidity and non-enzymatic browning were negligible. Loss of ascorbic acid was gradual at normal conditions and was sudden and rapid (82 and 60%) at accelerated storage conditions. Condition of storage and duration did not affect either total carotene or β -carotene. Chlorophyll in PBF decreased irrespective of storage condition and its duration (58 to 41%). Conditions of storage affected the sensory quality parameters including colour and appearance, aroma, taste and overall quality of the baby foods. However, these changes were slight at normal compared to accelerated condition. Period of storage also influenced these changes.

Processing, packaging temperature and duration of storage have been found to influence certain physical, chemical and sensory profiles of foods. High rates of diffused darkening and extensive browning have been found to occur in stored fruit purees^{1,2}. These colour changes were more pronounced at elevated temperatures of storage and on prolonged storage. However, storage did not hasten changes in titratable acidity, pH, moisture content, peroxide value, etc. and in fact, these changes were reported to occur more slowly³. Loss of ascorbic acid (50-70 per cent) and β -carotene (10-20 per cent) in processed fruit and vegetable purees, during storage have been reported^{4,8}. Loss of B-vitamins was dependent on temperature and time of storage; the higher the temperature and longer the storage, greater was the loss⁹⁻¹¹. Among the sensory parameters, flavour was found to get affected more on storage². Studies on vegetable based infant foods indicated that flavour was retained in stored foods¹².

Strained baby foods were prepared from carrots and green peas, which are highly nutritious. Changes in the physico-chemical and sensory parameters of these foods were evaluated under different conditions of storage for 180 days.

Materials and Methods

Strained carrot baby food (CBF) and strained green pea baby food (PBF) processed at 115.5°C for 59 and 61 min respectively, in 301 × 206 size cans were used for the study. The details of preparation and processing are described elsewhere^{13,14}. These

products were in 'Ready to serve' (RTS) form.

Storage conditions: The processed CBF and PBF were stored for 180 days at: (a) 27°C and 65 per cent RH (normal storage condition), and (b) 38°C and 92 per cent RH (accelerated storage condition). A few cans were stored at 4°C to serve as control. Stored cans were periodically evaluated at intervals of 0,45,90,135 and 180 days for physico-chemical and sensory changes.

Analytical methods: Moisture and titratable acidity were estimated by standard AOAC methods¹⁵. Ascorbic acid, β -carotene and total carotene were estimated by AVC methods¹⁶. Chlorophyll was estimated according to Comar¹⁷. Non-enzymatic browning (NEB) was expressed as colour absorbance at 420 nm of 60 per cent alcoholic extracts of CBF. The 60 per cent alcoholic extracts of PBF were extracted with benzene to avoid interference of colour¹⁸. Vacuum in the cans was determined using a pressure gauge as described by Ranganna¹⁸. Gross weight of the cans was noted before opening them for further chemical and sensory analyses.

Sensory evaluation: Two sets of discriminative - communicative (DC) panel each consisting of ten mothers among the scientific staff of the institute were chosen to evaluate the CBF and PBF during storage. The panelists were trained to perceive the slightest differences in the quality with samples in which changes were induced. Their consistency was tested in few repeat evaluations. The evaluation of the stored CBF and PBF was carried out at regular intervals in two phases. In the first phase, the overall quality of

TABLE 1. CUT-OUT ANALYSIS OF CANNED STRAINED CARROT BABY FOOD STORED AT DIFFERENT STORAGE CONDITIONS AND PERIODS

Storage temp/R.H.	Storage period (days)	Vacuum	pH	Gross wt. (g)	Quality observations			
					Colour	Consistency	Aroma	Taste
4°C (Control)	45	12	5.5	230	Yellowish orange	Smooth; free flowing	Typical	Sweet
	90	10	5.3	232	"	"	"	"
	135	10	5.1	240	"	"	"	"
	180	10	5.0	240	"	"	"	"
27°C/65 % RH (Normal)	45	12	5.4	240	Yellowish orange	Smooth; free flowing	Typical	Sweet
	90	10	5.0	245	"	"	"	"
	135	10	4.8	240	"	"	"	"
	180	10	4.6	240	"	"	"	"
38°C/92 % RH (Accelerated)	45	10	5.1	246	Yellowish orange	Smooth; free flowing	Typical	Sweet
	90	8	4.8	248	"	"	"	"
	135	8	4.6	240	Slight brownish orange	Smooth; free flowing	Mild	Bitter
	180	8	4.3	248	"	"	"	"

the reference and samples stored at normal and accelerated conditions was rated on a 5 point scale very good to poor; and in the second phase, individual quality attributes such as colour and appearance, consistency, aroma and taste were rated by multiple sample difference test. The panelists were asked to rate the individual quality attributes of the test samples compared to reference as 7 – intensity strong, superior to reference; 4 – no intensity difference compared to reference and 1 – intensity strong, inferior

to reference. The reference sample was also evaluated as one of the coded samples. The panelists were asked to comment on the quality deterioration of the test samples. They were also asked to rinse their mouth in between tasting different samples and to pause a few minutes to eliminate carry over taste, if any.

Statistical analysis: The chemical and sensory quality data for certain chemical constituents and sensory quality data for individual quality attributes and overall quality of CBF and PBF were analysed for

TABLE 2. CUT-OUT ANALYSIS OF CANNED STRAINED GREEN PEAS BABY FOOD STORED AT DIFFERENT STORAGE CONDITIONS AND PERIODS

Storage temp/R.H.	Storage period (days)	pH	Vacuum	Quality observations			
				Colour	Consistency	Aroma	Taste
4°C (Control)	45	7.5	12	Slightly greenish yellow	Smooth; and free flowing	Typical	Typical
	90	7.4	12	"	"	"	"
	135	7.2	14	"	"	"	"
	180	7.1	14	"	"	"	"
27°C/65% RH (Normal)	45	7.5	12	Yellowish green	Smooth; and free flowing	Typical	Typical
	90	7.2	10	"	"	"	"
	135	6.9	12	"	"	"	"
	180	6.7	12	Slightly yellow	"	mild	slight off taste
38°C/92% RH (accelerated)	45	7.2	14	Slightly yellowish green	Smooth; and free flowing	Typical	Typical
	90	7.0	10	"	"	"	"
	135	6.5	14	Slightly yellow	Tompy	Mild	Mild off taste
	180	6.1	10	"	"	"	"

Gross weight of cans at all conditions and periods of storage was 170 g.

difference due to effect of storage periods and storage conditions by analysis of variance appropriate to 4×3 factorial design with replications (3 for chemical analysis and 10 for sensory evaluation), followed by Duncan's new multiple range test^{19,20}.

Results and Discussion

Effect of storage on quality parameters: Data on the cut-out analysis of CBF and PBF during storage are shown in Tables 1 and 2. The cans did not show any marked changes in the vacuum during the 180 days of storage indicating the soundness of the hermetically sealed cans. A slight decrease in pH was noted in cans stored at normal storage condition in comparison with control samples; however, a rapid decrease in pH was noted in cans stored at accelerated storage condition. The drop in pH resulted in the reduction of green colour in PBF. This suggested that both the storage conditions and storage period influence the reactions involving slow but continuous release of organic acids in the product.

The quality of both the control and normally stored

samples remained unchanged at the end of 180 days. Changes in colour (darkening) and taste (bitterness) were markedly pronounced in the samples stored under accelerated storage condition at the end of 180 days of storage, a finding similar to that made by Livingston *et al*¹. Storage under accelerated condition caused distinct changes in colour and taste of PBF by the end of 135 days.

Effect on storage on certain chemical constituents: The effects of storage conditions and storage on chemical parameters were highly significant ($P \leq 0.01$). These data on CBF and PBF are presented in Tables 3 and 4. The change in moisture content in CBF and PBF during storage, was found to be statistically significant. The acidity in the stored CBF increased throughout the storage period, irrespective of the storage conditions. This increase in acidity correlated storage condition, the acidity almost trebled. This suggested that the release of organic acids continue to occur in foods during storage, the temperature of storage accelerating the rate of reaction. A similar trend was observed in stored cans of PBF. The rate of

TABLE 3. EFFECT OF STORAGE CONDITIONS AND STORAGE PERIODS ON CHEMICAL CONSTITUENTS FOR CANNED STRAINED CARROT BABY FOOD

Chemical parameters	Initial	Storage conditions			Storage periods (days)				SE(24 df)
		Control	Normal	Accelerated	45	90	135	180	
Moisture (%)	88.62	88.98 ^x	89.06 ^y	89.35 ^z	88.87 ^a	89.02 ^b	89.16 ^c	89.47 ^d	± 0.002
Acidity (%)	0.44	0.84 ^x	1.13 ^y	1.35 ^z	0.98 ^a	1.09 ^b	1.15 ^b	1.40 ^c	± 0.03
Non-enzymatic browning*	0.31	0.36 ^x	0.38 ^y	0.63 ^z	0.35 ^a	0.44 ^b	0.46 ^c	0.58 ^d	± 0.002
Ascorbic acid (mg%)	65.91	40.79 ^z	30.90 ^y	23.58 ^x	41.12 ^d	35.25 ^c	28.99 ^b	21.68 ^a	± 1.85
Total carotenes (mg%)	71.52	63.91 ^z	60.61 ^y	58.26 ^x	65.88 ^d	62.42 ^c	59.55 ^b	55.85 ^a	± 0.23
β-carotene (mg%)	68.29	62.47 ^z	58.57 ^y	55.43 ^x	64.08 ^d	60.22 ^c	57.77 ^b	53.23 ^a	± 0.09

*Colour absorbance at 420 nm.

SE (df) – Standard error of means (degrees of freedom)

Differences between overall means carrying different superscripts x,y,z for storage conditions and a,b,c,d for storage periods are significant ($P \leq 0.05$)

TABLE 4. EFFECT OF STORAGE CONDITIONS AND STORAGE PERIODS ON CHEMICAL CONSTITUENTS FOR CANNED STRAINED GREEN PEAS BABY FOOD

Chemical parameters	Initial	Storage conditions			Storage period (days)				SE(24 df)
		Control	Normal	Accelerated	45	90	135	180	
Moisture %	86.48	87.03 ^x	87.25 ^y	87.48 ^z	86.89 ^a	87.6 ^b	87.32 ^c	87.74 ^d	± 0.002
Acidity %	0.17	0.23 ^x	1.32 ^y	0.40 ^z	0.24 ^a	0.28 ^b	0.33 ^c	0.42 ^d	± 0.006
Non-enzymatic browning*	0.25	0.29 ^x	0.33 ^y	0.44 ^z	0.29 ^a	0.33 ^b	0.38 ^c	0.42 ^d	± 0.001
Ascorbic acid (mg%)	20.27	15.67 ^z	13.89 ^y	11.76 ^x	17.43 ^d	15.69 ^c	11.87 ^b	10.10 ^a	± 0.38
Chlorophyll (mg%)	31.16	21.40 ^z	19.78 ^y	16.37 ^x	22.61 ^d	19.74 ^c	17.72 ^b	16.65 ^a	± 0.16

*Colour absorbance at 420 nm.

SE (df) – Standard error of means (degrees of freedom)

Differences between overall means carrying different superscripts x,y,z for storage conditions and a,b,c,d for storage periods are significant ($P \leq 0.05$)

NEB was found to increase significantly with an increase in the storage temperature irrespective of the storage period in cans of both CBF and PBF. This was similar to the findings observed in guava puree stored at high temperatures².

Ascorbic acid was found to decrease throughout the period of storage. A gradual but significant ($P \leq 0.05$) loss of ascorbic acid was noted with prolonged storage time which was further influenced by temperature, similar to observations reported by Feaster²¹.

Total carotene content had also undergone considerable decrease ($P \leq 0.05$) in all samples stored at different conditions of storage and time. In control samples, the percent retention of total carotene was found to be more when compared to samples stored at

normal and accelerated conditions. A similar trend was observed in β -carotene content of stored CBF. The decrease in the retention of β -carotene content was found to be significant ($P \leq 0.05$) and was comparable to the 10 to 20 per cent loss of β -carotene in commercial baby foods stored for 3 years at 20°C, as reported earlier⁶. In general, the temperature and time of storage were found to significantly influence the retention of both total and β -carotene contents of stored CBF.

Per cent retention of chlorophyll was studied as an index of colour in PBF. Chlorophyll retention also showed a decreasing trend. The higher the storage temperature, lower was the retention of chlorophyll. The lowered retention of chlorophyll was found to be

TABLE 5. EFFECT OF STORAGE CONDITIONS AND STORAGE PERIODS ON SENSORY QUALITY FOR CANNED STRAINED CARROT BABY FOOD

Quality factors	Storage conditions			Storage periods (days)				SE (108df)
	Control	Normal	Accelerated	45	90	135	180	
Colour and appearance	3.8 ^a	3.8 ^a	3.5 ^a	3.9 ^y	3.9 ^y	3.5 ^x	3.5 ^x	± 0.13
Consistency	3.9 ^a	3.9 ^a	3.8 ^a	3.8 ^x	3.9 ^x	3.9 ^x	3.8 ^x	± 0.10
Aroma	4.0 ^b	3.7 ^b	3.2 ^a	3.4 ^x	3.9 ^y	3.9 ^y	3.5 ^{xy}	± 0.16
Taste	4.0 ^b	3.8 ^b	3.2 ^a	3.6 ^x	3.9 ^x	3.8 ^x	3.6 ^x	± 0.15
Overall quality	3.5 ^b	3.5 ^b	3.0 ^a	3.8 ^y	2.8 ^x	3.4 ^y	3.4 ^y	± 0.17

SE(df) - Standard error of means (Degrees of freedom)

Differences between overall means carrying different superscripts a,b for storage conditions and x,y for storage periods are significant ($P \leq 0.05$)

Limits for individual attribute means:

1.6 - 2.5 = Moderate intensity difference, inferior to control

2.6 - 3.5 = Slight intensity difference, inferior to control

3.6 - 4.5 = No intensity difference, equal to control.

Limits for overall quality means:

upto 1.5 = Poor (Not acceptable)

1.6 - 2.5 = Fair (Acceptable)

2.6 - 3.5 = Satisfactory

3.6 - 4.5 = Good

TABLE 6. EFFECT OF STORAGE CONDITIONS AND STORAGE PERIODS ON SENSORY QUALITY FOR CANNED STRAINED GREEN PEAS BABY FOOD

Quality factors	Storage conditions			Storage period (days)				SE (108 df)
	Control	Normal	Accelerated	45	90	135	180	
Colour and appearance	3.9 ^b	2.5 ^a	2.4 ^a	2.6 ^x	2.3 ^x	3.5 ^y	3.4 ^y	± 0.14
Consistency	3.8 ^a	3.0 ^a	2.7 ^a	2.6 ^x	3.1 ^{xy}	3.4 ^y	3.6 ^y	± 0.18
Aroma	3.8 ^c	3.2 ^b	2.7 ^a	2.5 ^x	2.9 ^x	3.7 ^y	3.8 ^y	± 0.16
Taste	3.8 ^c	3.1 ^b	2.4 ^a	2.5 ^x	2.7 ^x	3.7 ^y	3.6 ^y	± 0.16
Overall quality	3.6 ^b	2.8 ^a	2.4 ^a	2.5 ^x	2.6 ^{yx}	3.0 ^{y'}	3.2 ^{y'}	± 0.18

SE(df) - Standard error of means (Degrees of freedom)

Differences between overall means carrying different superscripts a,b,c for storage conditions and x,y,z for storage periods are significant ($P \leq 0.05$)

Limits for individual attribute means:

1.6 - 2.5 = Moderate intensity difference, inferior to control

2.6 - 3.5 = Slight intensity difference, inferior to control

3.6 - 4.5 = No intensity difference, equal to control.

Limits for overall quality means:

upto 1.5 = Poor (Not acceptable)

1.6 - 2.5 = Fair (Acceptable)

2.6 - 3.5 = Satisfactory

3.6 - 4.5 = Good

significant ($P \leq 0.05$) at all storage conditions and periods.

Effect of storage on sensory quality: In CBF, the effect of storage period was not significant with respect to consistency and taste irrespective of the storage conditions (Table 5). Similarly the storage conditions did not significantly alter colour and appearance and consistency, irrespective of the storage periods. Accelerated storage condition significantly affected the aroma of the product, with low overall mean score. The aroma changes between different storage periods were not clearly segregated as the overall mean scores were closer to the control. Whereas duration of storage did not alter the scores for taste, condition of storage did influence it to a considerable extent. At accelerated storage condition, the overall mean score was slightly inferior to the control sample, while the sample stored at normal condition was closer to the reference. Storage at accelerated condition and its duration were found to affect the overall quality of the sample.

The mean scores of sensory evaluation of PBF at different storage conditions and periods were found to be highly significant ($P \leq 0.01$) in all individual quality attributes and overall quality (Table 6). The colour and appearance of the samples stored at normal condition were slightly inferior to controls while the samples stored under accelerated condition were moderately inferior. Upto 90 days, colour and appearance did not significantly differ among samples. Consistency scores of samples stored at normal and accelerated storage conditions were comparable but were slightly inferior to those of controls. The trend observed for aroma and taste in samples stored at different conditions were similar. The overall quality is definitely influenced by storage conditions. However, changes in overall quality due to storage period were not very clearly segregated. The low and inconsistent scores obtained for sensory quality parameters were due to the changes in chlorophyll and ascorbic acid content of PBF on storage.

Prolonged storage and conditions of storage were found to cause a gradual loss of ascorbic acid in the stored CBF and PBF. Total and β -carotene contents of CBF were found to be fairly stable during storage. Retention of chlorophyll in PBF was low with storage. Accelerated storage had lowered all the sensory quality parameters.

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BIOCHEMICAL CHANGES AND PATULIN AND TERREIC ACID PRODUCTION BY *ASPERGILLUS TERREUS* IN DIFFERENT CULTIVARS OF MAIZE (*ZEA MAYS* LINN.)

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Biochemical changes and patulin and terreic acid production by *Aspergillus terreus* in different cultivars of maize (*Zea mays* Linn.) was investigated. Infestation of maize seeds with *A. terreus* increased phenols, reducing sugars, proteins and free fatty acids production except in 'NMH-10' where a decreasing trend was observed. However, free amino acids decreased in infected seeds of all the cultivars except in white cultivar in which free amino acids increased considerably. White cultivar of maize supported least amount of both patulin and terreic acid production by *A. terreus*. On the other hand, 'NMH-10' cultivar was highly susceptible to *A. terreus* infection and supported maximum amount of patulin and terreic acid production.

Recently, exploitation of genetical resource has been advocated by several workers including Priyadarshini and Tulpule¹, Bilgrami *et al*². and Zuber *et al*³, for the control of mycotoxin contamination in agricultural commodities. It was, therefore, felt worthwhile to screen different cultivars of maize for their resistance to proliferation and patulin and terreic acid production by *A. terreus*.

Surface sterilized seeds (200 g) of different cultivars of maize ('Local', 'Pioneer', 'MMH-1', 'NMH-10', 'DDH', 'DHM-103', 'Charminar' and 'White') were

inoculated with 7-day old *Aspergillus terreus* grown on cellophane implanted malt agar plates after air drying under laboratory conditions (27-29°C and 60-70 RH) for 30 days. At the end of the incubation period, the infested seeds were analysed for reducing sugars⁴, proteins⁵, total phenols⁶, free amino acids⁷ and free fatty acids⁸ by employing standard methods.

The patulin and terreic acid from infested maize seeds were extracted and estimated as described by Subramanian⁹ and Subramanian *et al*¹⁰, respectively.

Table 1 reveals that the infestation of maize seeds by *A. terreus* caused significant biochemical changes which, however, varied with the cultivar. In general, reducing sugars increased due to the infestation by *A. terreus*. The increase was maximum in local cultivar and minimum in 'Charminar' cultivar. On the other hand, reducing sugars were depleted in 'NMH-10' which may be attributed to the assimilation by the infesting fungus. It was intermediate in rest of the cultivars. Similarly, protein content also increased due to *A. terreus* infestation in all the cultivars except in 'MMH-1' and 'NMH-10', where a decrease was noted. The protein content remained unaltered in white variety infested with *A. terreus*. The increase in protein content may be due to the addition of fungal proteins¹¹, while the decrease could be attributed to the assimilation of host proteins by the fungus¹². Free amino acids decreased considerably due to *A. terreus* infestation. This decrease was more in pioneer and local cultivars. However, in white cultivar free amino acids increased significantly. The accumulation of free amino acids may be one of the defence acts of the host. Similarly, Singh and Prashar¹³ have recorded an accumulation of free amino acids in resistant variety of peach fruits to *Rhizopus stolonifer* infection. The free fatty acids increased under the influence of *A. terreus*

TABLE 1. BIOCHEMICAL CHANGES IN DIFFERENT CULTIVARS OF MAIZE SEEDS INFESTED BY *ASPERGILLUS TERREUS*

Cultivar	Phenols (mg/g)		Reducing sugars (mg/g)		Proteins (mg/g)		Free amino acids (mg/g)		Free fatty acids (mg/100 g)	
	Hlthy	Inf	Hlthy	Inf	Hlthy	Inf	Hlthy	Inf.	Hlthy	Inf
Local	0.40	0.80	75	180	31.0	37.0	0.58	0.11	0.61	0.72
Pioneer	0.55	1.30	40	105	47.0	72.2	0.50	0.10	0.43	0.98
MMH-1	0.40	0.75	40	70	39.0	32.5	0.36	0.34	0.35	0.86
DDH	0.35	1.05	55	75	50.5	58.5	0.52	0.20	0.36	0.97
NMH-10	0.60	0.60	90	72	62.5	47.5	0.66	0.60	0.39	0.96
DHM-103	0.70	0.75	55	80	39.0	66.5	0.54	0.38	0.35	0.90
Charminar	0.45	0.95	75	80	29.5	74.5	0.46	0.28	0.40	0.67
White	0.74	0.84	80	120	35.5	36.0	0.56	1.02	0.38	0.51

Hlthy = Healthy; Inf = Infected

TABLE 2. PRODUCTION OF PATULIN AND TERREIC ACID BY *A. TERREUS* IN DIFFERENT CULTIVARS OF MAIZE SEEDS

Cultivar	Patulin (ppb)	Terreic acid (ppb)
Local	13	29
Pioneer	14	37
MMH-1	20	43
DDH	22	36
NMH-10	26	53
DHM-103	23	52
Charminar	18	47
White	8	15

which was more marked in 'DHM-103', 'NMH-10', 'DDH' and 'MMH-1' cultivars and minimum in local variety. The phenol content also increased considerably in 'DDH' and 'Pioneer' cultivars.

The amount of patulin, a carcinogen¹⁴ and terreic acid, a hepatotoxin¹⁰ production by *A. terreus* in different cultivars of maize varied significantly (Table 2). The white cultivar supported least amount of patulin and terreic acid, while 'NMH-10' supported maximum. Local and 'Pioneer' cultivars also supported considerably low amount of patulin and terreic acid production. Such variation in supporting the production of aflatoxins by different cultivars of maize was also reported by Zuber *et al*¹⁵. and Bilgrami *et al*².

From the present investigations, it is clear that patulin and terreic acid production varied with the cultivar of maize. Therefore, exploitation of the genetic resource for cultivating patulin and terreic acid free maize is desirable. Screening of large number of maize cultivars so as to select a maize cultivar with all good agronomic characters in addition to resistance to patulin and terreic acid production is desirable.

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EFFECT OF PARTICLE SIZE ON PROCESSING OF GOAT MEAT PATTIES

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Comminution as a means of particle size reduction in processing of goat meat patties has been studied. A comparison of comminution methods indicated that the patties processed from coarse minced meat (passing through 9 mm plate of the meat grinder) had reduced desirable appearance, juiciness, texture and overall acceptability scores. Desirability of patties increased as the particle size decreased. Sensory panel scores for overall palatability were highest for the one coarse + one fine mince (4 mm plate) followed by 2 min chopping in bowl chopper.

Various methods of comminution have been employed for processing of ground meat products which include mincing, chopping, slicing and flaking. The effect of particle size on characteristics of beef products using fine, medium and coarse flaked meat has been studied in developed countries¹. In India, most of the comminuted meat products are processed by meat mincer. Meat cutter (bowl chopper) is gradually being introduced in meat processing. However, no information is available on the effect of comminution methods on product characteristics. In the present investigation, effect of comminution methods and time on quality of goat meat patties were investigated.

Lean and fat meat were collected from one year old female goats slaughtered at the Institute and were chilled for 24 hr at 4°C. The comminution schedule adopted for lean was as follows:

- A : One coarse mince (9 mm plate),
- B : One coarse mince + one fine mince (4 mm plate),
- C : One coarse mince + two fine mince,
- D : One coarse mince + one fine mince + 2 min chopping (Hobarts 84181 D model),
- E : One coarse mince + one fine mince + 4 min chopping.

Fat was minced through a 4 mm plate of the meat grinder.

Goat meat patties were made using 90 parts of meat (having lean to fat ratio of 80:20), 1 part ground spice mix, 2 parts salt and 7 parts added water. All the ingredients were mixed thoroughly using an electrically operated meat mixer (Hobarts N-50). Doughs weighing 100 g each were moulded into patties having 87 mm diameter and 18 cm height. Patties were broiled in an oven at 190°C for 15 min to get an internal temperature of 74±2°C. The temperatures were recorded by using a probe type thermometer (Wahl Heat-Probe Thermometer, model-2000). The broiled patties were weighed. Two diameters were measured perpendicular to each other and two heights were recorded from the opposite ends of a diameter of each broiled patty with the help of vernier caliper. Warm patties were evaluated for general appearance, flavour, texture, juiciness and overall acceptability on a 7-point Hedonic scale. Warner Bratzler shear press was used to record the shear force value on 1.27 cm² slabs, prepared by removing the outer crust of the broiled patties, after 24 hr chilling at 4°C. Proximate composition of broiled patties was analysed according to AOAC methods². All data were analysed statistically³.

Results on processing yield, diameter changes and shear properties of goat meat patties are presented in Table 1. One coarse (9 mm plate) plus one fine (4 mm plate) mincing followed by 2 min run in bowl chopper gave the highest processing yield of 77.50 per cent.

TABLE 1. YIELDS, SHEAR VALUES AND DIMENSIONAL CHANGES IN BROILED GOAT PATTIES PROCESSED BY DIFFERENT COMMUNITION METHODS

Parameters	Comminution method				
	A	B	C	D	E
Yield (%)	68.23 ± 2.49 ^a	70.65 ± 2.46 ^a	72.17 ± 2.85 ^a	77.50 ± 2.69 ^a	73.06 ± 2.96 ^a
Shear value (kg/1.27 cm ² slab)	2.64 ± 0.19 ^c	1.86 ± 0.11 ^b	1.49 ± 0.07 ^a	1.53 ± 0.06 ^a	1.52 ± 0.04 ^a
Diameter (mm)	67.50 ± 1.04 ^a	69.86 ± 1.84 ^a	68.29 ± 1.58 ^a	69.14 ± 1.61 ^a	65.14 ± 1.55 ^a
Height (mm)	23.83 ± 1.12 ^a	22.93 ± 0.61 ^a	24.29 ± 0.76 ^{ab}	27.21 ± 0.96 ^b	28.57 ± 0.99 ^b

Values are means ± S.E.

Means with same superscript in each row do not differ significantly (P<0.05)

TABLE 2. SENSORY SCORES OF BROILED GOAT MEAT PATTIES

Parameters	Comminution method				
	A	B	C	D	E
General appearance	4.09 ± 0.28 ^a	5.09 ± 0.21 ^b	5.27 ± 0.24 ^b	6.00 ± 0.27 ^c	5.45 ± 0.25 ^{bc}
Flavour	4.91 ± 0.28 ^a	5.45 ± 0.25 ^{ab}	5.45 ± 0.25 ^{ab}	5.91 ± 0.28 ^b	5.55 ± 0.25 ^b
Texture	3.82 ± 0.35 ^a	5.00 ± 0.27 ^b	5.82 ± 0.26 ^c	6.55 ± 0.21 ^c	5.73 ± 0.27 ^{bc}
Juiciness	4.64 ± 0.31 ^a	5.09 ± 0.21 ^{ab}	5.82 ± 0.30 ^{bc}	6.09 ± 0.37 ^c	5.55 ± 0.25 ^{bc}
Overall acceptability	3.64 ± 0.29 ^a	5.00 ± 0.19 ^b	5.82 ± 0.33 ^c	6.55 ± 0.25 ^d	5.64 ± 0.15 ^{bc}

Values are means ± S.E.

Means with same superscript in each row do not differ significantly (P<0.05)

TABLE 3. PROXIMATE COMPOSITION OF BROILED GOAT MEAT PATTIES

Parameters	Comminution method				
	A	B	C	D	E
Moisture (%)	58.88 ± 0.73 ^a	50.49 ± 0.65 ^a	59.07 ± 0.59 ^a	58.77 ± 0.37 ^a	57.58 ± 0.19 ^a
Protein (%)	21.87 ± 0.17 ^a	21.10 ± 0.12 ^a	21.68 ± 0.17 ^a	21.60 ± 0.22 ^a	21.46 ± 0.24 ^a
Ether extract (%)	11.49 ± 0.25 ^a	12.81 ± 0.51 ^b	13.73 ± 0.40 ^{bc}	14.12 ± 0.25 ^c	14.77 ± 0.19 ^c

Values are means ± S.E.

Means with same superscript in each row do not differ significantly (P<0.05)

The minimum yield of 68.23 per cent was recorded in single coarse minced patties. The treatment effect was not statistically significant in respect of cooking yield and diameter changes. Shear value was significantly higher for coarse minced patties indicating their maximum toughness. Tenderness increased with the decrease in particle size. This was expected as finer mincing and chopping by bowl chopper would reduce the fascia and other connective tissue to smaller particle size. Higher shear values have also been reported for reformed beef steaks made from coarse ground as compared to fine ground meat⁴.

Sensory scores for goat meat patties made from different particle sizes are presented in Table 2. The scores for general appearance, flavour, texture, juiciness and over acceptability were highest for patties made by one coarse plus one fine mincing followed by 2 min chopping in bowl chopper. Single coarse minced patties had the lowest scores for all the parameters. These patties had large chunks of meat and fat giving a more coarse texture than would normally be considered acceptable. Very coarse particle size reduces the normal appearance and overall acceptability of the finished product⁵.

The proximate composition of goat meat patties is presented in Table 3. The moisture content of patties ranged between 58 and 59% but no significant differences were recorded among comminution

methods either for moisture or protein content. Earlier, it was also reported that comminution method generally had no effect on moisture content of the processed product⁴. The patties with maximum reduction in particle size had maximum fat retention. The fine cutting and processing in bowl chopper probably emulsified the fat to a certain extent resulting in better retention during cooking.

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BOOK REVIEWS

Chemical changes during Food Processing: Volume 1: Proceedings of the IUFOST International Symposium Nov 1984, Instituto de Agroquímica y Tecnología de Alimentos C/ Jaime Roig, 11 46010 Valencia. Spain; Pp: 373; Price: \$50 or 6000 pts.

The symposium – Chemical Changes in Food during Processing – known by the acronym MOCCA symposium – held in Valencia, Spain from November 5-7, in 1984 was composed of three groups – A. Chemical changes during processing, B. Analytical methods to study the chemical changes. and C. Current topics in food science and technology. The present volume relates to chemical changes which were discussed in six sessions. Each session has a state of art paper followed by a few research papers in the area.

Session A1 relates to the effect of thermal processing, cooking and culinary practices on the quality and nutritional value of animal and fish foods except for a paper on the interaction between sunflower proteins and oxidised lipids.

In the second session on the processing changes in animal proteins, the negative and the positive effects of various methods of preservation like chilling, freezing, thermal processing, removal of moisture, ionizing radiation and measures for control of undesirable changes have been discussed in the paper on organoleptic aspects of processing changes in meat proteins. The role of myofibrillar and sarcoplasmic proteins on emulsifying capacity and water solubility; effect of electrical stimulation and quick chilling on alterations in myoglobin, oxymyoglobin and metmyoglobin and their effect on the colour of chilled meat have been discussed. Extra low-voltage stimulation of bovine carcass has shown no significant effect on quality.

Salting, pickling, heat treatment and drying steps in the preparation of cured pork caused changes in pH, redox potential, reducing substances and water activity (a_w) which influence the biochemical reactions and microbial development. Chemical changes in proteins during slow and rapid maturation processes showed that total extractable nitrogen which accounted for 40% of total nitrogen in ham decreased during refrigeration, while non-protein nitrogen increased almost proportionately. Though rapid cured meat developed typical sensory characteristics in the slow process, it increased with maturation upto 12 months.

Changes in neutral lipids (NL), free fatty acids (FFA), polar lipids (PL), peroxide value and total

carbonyl compounds (TCC) in adipose subcutaneous tissue and in the lean of ham during slow and rapid maturation of ham and their effect on the development of aroma form the last paper of the second session.

Session A3 relates to chemical changes in processing of fermented foods. Bread dough fermentation is the most important step in the ultimate quality of bread. Weight loss, changes in chemical composition, carbohydrates, phytate, lipids, nitrogen and flavour during dough fermentation step has been critically reviewed. Also included in the session are six research papers of which four relate to changes in total lipids, their fractions and individual components in the dough and in the liquid phase of the dough, dietary fiber fractions, and volatile organic acids (C_2-C_5) during bread dough fermentation. Cabrales cheese, manufacture by artesanal methods in small dairies situated in some areas of Spain is made from cow's milk or partly substituted with sheep or goat's milk. Mineral composition of cheese which is dependent on Ca: P ratio and the manufacturing method form the subject matter of another paper. Yet another paper in the session shows that tryptophol content in wines is more dependent on the type of yeast and the vinification method.

In session 4, on processing changes in fruit and fruit juices, preparation and preservation of fruit juices and concentrates particularly from orange for subsequent manufacture of products and methods for achieving stability during storage have been critically reviewed. Two research papers in the session relate to the role of pectic enzymes in the cloud loss of citrus juices and softening of texture of canned apricots. Another paper is on changes in different fractions of pectic substances particularly during blanching of peas which are significant as compared to changes during freezing or frozen storage. Changes in 10 volatile components of the 109 components isolated from verna orange juice as a result of cold and hot filling and during storage have been examined. Malto-dextrin added to the extent of 30% has been shown to aid in the spray drying of 'horchata', a beverage prepared by extracting the tuber "Chufa" from the plant *Cyperus esculentus* L. Effect of SO_2 on texture of apricots and peaches have been studied using Instron Texturometer and SEM.

The 3 papers of session A5 relate to chemical changes during brine curing of olives and cucumber brought about by lactic acid bacteria and the changes during ripening of olives brought about by cellulolytic

enzymes which sometimes cause the breakdown of cellular structure.

In the last session (A6), the compositional features of fruits which are important from the point of view of consumer acceptability and nutritive value which lead to the descriptive characteristics of the fruit, metabolic and compositional changes during injury, storage and ageing, and control of compositional changes have been discussed. Research papers presented show that peroxidase has no role in the colour change of orange skin during ripening; and sucrose is an adequate index to fix the harvest date of Blanquilla pears while glucose level is a good index to study the interaction between harvest date and storage conditions.

The lead papers of each session present an excellent review of the state of art and some of the research papers have interesting findings. In spite of the fact that most of the authors are of Spanish origin, the presentation does not suffer from clarity. This is a very useful reference book for research workers and students of food science and technology.

S. RANGANNA
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Chemical Changes During Food Processing, Vol.II: Proceedings of the IUFOST International Symposium; Nov 1984, Institute de Agroquimica y Tecnologia de Alimentos, C/ Jaime Roig, 11 46010 Valencia, Spain; pp.149; Price: 50 or 6000 pts.

The processing of foods affects the physico-chemical, biological and nutritional aspects of various food constituents. A Symposium covering this area was organised in 1984 in Valencia by CSIC Spain in association with the International Union of Food Science and Technology and the proceedings have now been published. Topics dealt in Volume II of the proceedings specially emphasise the methodological aspects in relation to food enzymes, polysaccharides such as starch and pectin, lipid-protein interactions, protein quality, effect of processing on meat and fish proteins and nature of microorganism in relation to fermented foods.

Each of these areas is reviewed by an expert in the field and the literature citations in the articles are of recent years. The article by Rhodes on food enzymes has various sections: the use of exogenous enzymes to improve the quality of food and food ingredients, endogenous enzymes in relation to quality of foods of plant origin, modern methods of isolation of enzymes and enzyme activity in relation to food quality. Developments in the methodology for the identification of mono, oligo and polysaccharides in

food materials has been reviewed by Mercier with special reference to HPLC, affinity chromatography, enzyme electrodes and near infrared reflectance spectroscopy. The analytical methods used to monitor polysaccharide changes during fruit and vegetable processing have been reviewed by a group of the Agricultural University at Wageningen, Holland.

The interaction of oxidised lipids with proteins has major implications on the quality and shelf life of food products. The methodology to monitor these changes by various techniques has been reviewed by Nielson. Meat products constitute an important part of the Western diet and the effect of processing on meat quality determines its acceptability. Methodology to evaluate meat protein characteristics as affected by processing are reviewed by Honikel and Hamm of West Germany. Textural quality of flesh foods are largely determined by the properties of the major constituent proteins myosin and actin. Recent advances in analytical methods for these proteins in relation to fish have been reviewed by Mackie of Torry Research station, Aberdeen.

The proceedings contain a substantial amount of very useful information of value to all food scientists and technologists. I recommend these proceedings to all investigators in the food science field who need to acquaint themselves with recent developments in the analytical methodologies related to food processing.

D. RAJAGOPAL RAO
C.F.T.R.I., MYSORE:

Low Digestibility Carbohydrates: Proceedings of the TNO-CIVO Workshop held on 27-28th November, 1986, Zeist, The Netherlands. Published by Pudo, Wageningen, 1987; Pp:148; Price: Dfl 60.00

The above workshop was organized in honour of Dr. A.P. de Groot on the occasion of his retirement as head of the department of biological toxicology, TNO-CIVO. The subject of low digestibility carbohydrates (LDC) was chosen 'to pay tribute to the scientist and person who has contributed a lot in this area'.

Low-digestibility carbohydrates are different group of products like sugar, alcohol, cyclodextrins, chemically modified starches or dietary fibre. One common feature is that all of them are incompletely digested. They are partly digested by microflora in the large intestines and give rise to some typical physiological effects.

The proceedings of the workshop cover a general overview of LDC in foods, their nutritional, toxicological effects and regulatory aspects.

Proceedings also include a forum discussion on the effects of LDC in experimental animals and the relevance of these results for man.

The first part of the proceedings deals with chemical and biochemical aspects, structural features and mechanisms underlying the phenomenon of LDC. Biological effects induced in rats by high levels of LDC and the chemistry of microbial aspects upon animal utilization of LDC have been discussed. An overview of starch and its derivatives used in food industry, includes world production figures, types available and their application.

In the second part, the major emphasis is on the toxicological aspects of sugar alcohols, like xylitol, lactitol, and isomalt. Results obtained on both experimental animals and humans have been presented.

The next part is devoted to results of studies on dietary fibre on utilization of minerals and protective effects of dietary fibre on colon carcinogenesis in rats. Recent trends in research on slow release carbohydrates in relation to nutritional management of diabetes are discussed. This section also includes a review on degradation of LDC in the GI tract and their energy value.

The topic of the forum discussion was: "Is there a common mechanism underlying the effects observed with low digestible carbohydrates in animal studies and how relevant are these effects for man?". Experts from Switzerland, The Netherlands, USA, UK participated in this discussion.

The last part of the proceedings presents information on the regulatory aspects of LDC, and the conclusions of the FASEB (Federation of American Societies for Experimental Biology) expert panel on sugar alcohols and lactose.

The workshop ended with some general thoughts on future research.

The "proceedings" has been edited by D.C. Leeg Water, V.J. Ferow and R.J.J. Hermus.

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"Foods" Facts and Principles: by N. Shakuntala Manay and M. Shadakshara Swamy, Wiley Eastern Ltd., 6, Shri B.P. Wadia Road, Basavanagudi, Bangalore – 560 004, 1987; pp. 524; Price: Rs.80/-

The first part of the book deals with food chemistry and provides with the basic information on chemistry of foods which is available in most books on food science or chemistry. In this section, chapters on flavours, enzymes and properties of foods are

particularly useful for students of Nutrition. The inclusion of physico-chemical constants in the chapter – lipids would have been a relevant addition to the students.

Details with regard to requirements of protein, vitamins and minerals need to be explained using discussions and suggestions as stated by Indian Council of Medical Research (ICMR).

Food and food products – the second part is the largest portion of the book and includes details with regard to various foods such as beverages, fruits, vegetables, cereals, cereal products, pulses, nuts, oils and fats in foods, spices, milk and milk products, poultry, sea foods, sugar and confectionery. Useful information on most of the Indian foods and their preparations are included.

In almost all chapters, relevant Indian food production figures are given. These relate to either 1982 or earlier years. Data for recent years are not available and it would have been appropriate to have included these.

contributed by Indian scientists with special emphasis could have been presented in the chapter on cereals.

The information given in the chapter on oils and fats in food does not quite fit the title. The statement 'oilseeds are also rich in proteins and oilseed cake obtained after the extraction of oils can be processed to produce protein rich foods. Such foods are in the market and have helped solve the protein deficiency of vulnerable sections of our population' – is not only out of tune with current concepts on the aetiology of the protein energy malnutrition but also out of place. The authors seem to have equated nutritional requirements with recommended allowances of fat as suggested by the ICMR. A paragraph or two setting out the views as presented by the ICMR should have put the matter in proper perspective.

In a book, written by Indian authors meant for Indian students, the use of Indian terminology of foods would have been more relevant and meaningful than Western terminologies. The use of terms such as okra, eggplant and finger millet are examples.

Several special processed products have been discussed in some of the chapters. A note on their availability in India would have been in order.

In the last part of the book – food preparation, preservation and processing, useful discussions have been presented on cooking of foods and food quality. A wide variety of food additives have been explained. Food preservation and processing methods are dealt in detail. The uses of additives and information on household methods of preservation relevant to Indian conditions have not received the emphasis they should have had.

This is perhaps the first comprehensive book on food science written by Indian authors. In the preface, it has been stated that this book is intended for use by honours and PG students. It can fulfil the needs of graduate students but its usefulness at the PG level seems to be limited.

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Water Activity – Theory and Applications to Food:

Edited by Louis B. Rockland and Larry B. Beuchat, Marcel Dekker Inc., New York and Basel; 1987; pp:404; Price: \$59.75 (U.S. and Canada) \$71.50 (all other countries).

The editors and authors have produced a valuable addition to the expanding literature on water activity and its relation to food quality and stability. Water activity is now considered a major food quality attribute which plays an important role in directing the nature of physico-chemical changes that occur in foods. The book is a collection of fifteen papers. The papers have brought an update of current literature on several aspects of water activity which influence enzyme activity, lipid oxidation, vitamin degradation, non-enzymic browning, pigment stability, protein denaturation, starch retrogradation and textural changes in foods. The combined effect of temperature, water activity, pH and redox potential affecting the stability of foods based on meat has also been discussed in one of the papers. Other papers of interest are stabilization of surface of intermediate moisture foods and the use of water activity in food formulation.

The apparatus and methodology adopted for collecting water activity-moisture content data must satisfy a standard without which no meaningful conclusion can be drawn from the analysis of the data. Experiments conducted in some European research laboratories have resulted in development of such a standard and it is discussed in one of the papers.

Adaptation, growth, toxin production, sporulation and death of microorganisms as affected by water activity have been discussed in some of the papers. A paper on media requirement for detection and enumeration of microorganisms adapted to low water activity environment is an important inclusion to the book. Nuclear magnetic resonance method of finding the structure and dynamic characteristics of water present in food is also a valuable addition. The temperature dependence of water activity and thermodynamic considerations for finding the nature

of water binding at the molecular level are described in two papers.

There are a few minor snags at some places of the book. The symbols used in some of the equations in the first paper of the book have not been adequately explained. The use of the phrase 'salt slush' would have been avoided by using 'saturated salt solution' in the twelfth paper of the book. In spite of these, the book on the whole is well written and is worthy of possession in institutional libraries.

H. DAS
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Recommendations for the Processing and Handling of Frozen Foods: International Institute of Refrigeration 177, Boulevard Malesherbes, F-75017, Paris (France; 1986; pp: 418; Price – not mentioned.

This 3rd edition of the 'Red Book' in the IIR series is an extension and revision of the 2nd edition which was widely accepted. The whole text of this volume was deliberated by the working party composed of many eminent authorities from various countries.

This book is precise and distinct in expression and is an easily readable document of valuable scientific and technical information. It can serve as features of freezing of foods.

In this edition, the chapters on 'handling food stuffs' and 'physical, biochemical and microbiological behaviour of products' have been expanded. The chapter on bakery and dough based products has been expanded. 'Microwave technology' has been introduced. Other chapters dealing with 'freezing technique' and 'handling cold stores' are relatively more brief.

First three chapters cover the general and special definitions of freezing, scientific aspects of freezing regarding physical, biochemical, microbiological and nutritional changes during freezing and storage. The factors which influence these changes in foodstuffs have been pointed out and control of these factors to minimise the spoilage and extend the storage life of foods has been discussed.

Chapter 4 deals with different types of freezing methods, systems, package system, transport of frozen foods, display methods and thawing and tempering processes. This section gives the recommendations for processes, methods and favourable conditions for the various stages involved in freezing and storage upto consumption. Limitations and suggestions to overcome the difficulties have also been pointed out.

Sections 5 and 6 present collective information on

gradual cumulative and irreversible loss of quality of frozen food with time which arises due to product, processing, packaging-factors and time, temperature, tolerance-factors and some guidance on relative storage characteristics in terms of practical storage life of common frozen foods available throughout the world. Chapter 6 gives the PSL of different frozen foods at different specified temperatures of storage.

In chapter 7 the various types of frozen foods including prepared and bakery products have been discussed in detail for raw material, treatments, packaging, freezing and storage. The coverage is good for each commodity but in brief. This section provides information, advice and recommended conditions based on present knowledge.

General advice, methods and procedures to save energy and reduce the cost of energy incurring during freezing technique, processes and storage have been brought to light in the final chapter. This section consists of valuable suggestions, ideas and energy saving measures for freezing operations and storage.

On the whole, this book will be very useful to many food technologists, engineers and cold storage holders for the adoption of rapidly advancing freezing technology and reducing the food losses incurred during preservation.

R. THIAGU
C.F.T.R.I., MYSORE



AFST (1) News

Bangalore Chapter

The Annual General Body Meeting was held recently and the following office bearers were elected for 1988-89.

<i>President</i>	: Dr. Muddappa Gowda
<i>Vice President</i>	: Mr. Devaraiyah
<i>Hon. Secretary</i>	: Dr. D.R. Ranganath
<i>Joint Secretary</i>	: Mr. Das
<i>Hon. Treasurer</i>	: Mr. Gururaja Rao

Nagpur Chapter

The Annual General Body Meeting was held on 8th April 1988. The following office bearers were elected for 1988-89.

<i>President</i>	: Shri A.K. Bhiwapurkar
<i>Vice President</i>	: Dr. S.D. Bhalerao
<i>Hon. Secretary</i>	: Shri D.K. Kawadkar
<i>Jt. Secretary</i>	: Shri I.H. Ali
<i>Hon. Treasurer</i>	: Dr. G.V. Mulmuley

Madras Chapter

The Annual General Body Meeting was held on 6th May 1988. The following office bearers will be continued for the year 1988.

<i>President</i>	: Dr. T.S. Santhanakrishnan
<i>Vice President</i>	: Dr. P.G. Adsule
<i>Hon. Secretary</i>	: Shri K.L. Sarode
<i>Hon. Treasurer</i>	: Shri N. Ibrahim
<i>Hon. Jt. Secretary</i>	: Shri K. Manoharan
<i>Editor</i>	: Smt. Malathi Mohan

Annual General Body Meeting 1987

The Annual General Body Meeting of the Association for the year 1987 was held on 3rd June 1988 at New Auditorium, U.D.C.T., Matunga, Bombay. The highlights from the Secretary's Report are mentioned below.

The membership of the Association at present stands at 2229 and has crossed the coveted 2000 mark indicating the increasing stature of the Association as a truly professional body.

IFCON 88.

The major event during the year 1988 was the successful holding of the II International Convention and FOOD EXPO at Mysore during 18 - 24th February 1988. Several organisations like National Dairy Development Corporation, Agricultural & Processed Food Products Exports Development Authority, M/S. Parle Exports, Food Specialities,

M/S. Lipton India, M/s. Brooke Bond, M/s. Kejriwala Enterprises and some of our own chapters came forward to help by way of financial support and donations. The Convention was inaugurated on 18th February 1988 at Kalamandira, Mysore by Sri. R.V. Deshpande, Minister for Agriculture, Government of Karnataka.

The distinguished Nuclear Scientist Dr. Raja Ramanna, Chairman, Atomic Energy Commission in his presidential address stressed the historical importance of Food and its traditional base particularly in developing nutritious foods and suggested in depth study of the same.

The convention was attended by delegates from 24 countries viz. USA, Denmark, UK, Switzerland, Sweden, Japan, Singapore, China, Bangladesh, Mexico, Brazil, Austria, Thailand, Nepal, Nigeria, Korea, Federal Republic of Germany, Sri Lanka, Poland, Egypt, Hungary, Greece, Honkong and India.

The technical programme covered nearly 27 areas in the field of food science, and technology as well as related subjects like sociology, anthropology etc. In all, 151 papers were presented focussing on the various aspects. A total of 400 poster papers were presented by young scientists. About 1000 registered participants and several invitees attended the convention.

The FOOD EXPO patronised by many leading industries was inaugurated by Dr. Raja Ramanna on 18th February 1988. It was attended not only by the delegates but also by the general public. On this occasion, the publications entitled "Food Technology Overview" and "Abstracts of Papers" were brought out and released.

AFST (I) Awards for the year 1987

1. Prof. V. Subrahmanyam Industrial Achievement Award:- This award was shared by Sri N.A. Pandit, a well-known Food Technologist, Bombay and Dr. T.R. Sharma, Director, Defence Food Research Laboratory, Mysore.
2. Laljee Godhoo Smarak Nidhi Award:- Dr. Susanta Kumar Roy, Senior Scientist/Project Co-ordinator, Post-harvest Technology of Horticultural Crops, IARI., New Delhi for the year 1986 and Dr. A.M. Nanjundaswamy, Scientist, Fruit & Vegetable Technology, CFTRI., Mysore for the year 1987.

3. **Best Student Award:**– Ms. Juhi Raikhy, Student of M.Sc. (Food Science & Technology), College of Agriculture, PAU., Ludhiana.
4. **Suman Food Consultants Travel Award:**– Ms. Riyasur, Student of M.Sc. (Food Technology), Department of Food Science & Technology, PAU., Ludhiana.
5. **Gardners Award:**– Dr. R. Chinnaswamy & Dr. K.R. Bhattacharya, Scientists, Grain Science &

Technology, CFTRI., Mysore. This award was for their paper published in the Journal of Food Science and Technology Vol. 23 No.1, pp 14-19 entitled “Pressure parboiled rice; a new base for making expanded rice”.

On this occasion, the past and present editors of Journal of Food Science & Technology were honoured as the journal is in its 25th year of publication.

SUMAN FOOD CONSULTANTS TRAVEL AWARD

This award is instituted in the name of M/s Suman Food Consultants, New Delhi, to be awarded to a student pursuing Post Graduate Degree/Diploma courses in Food Science/Technology in any recognised University in India.

The Award will be decided based on the best essay to be submitted by the applicant on 'Flavour Development in Heat Processed Foods'. Five copies of the essay containing 15-20 pages (A4 size) of typed matter with appropriate bibliography and a certificate from the head of the department under whom the student is working should be enclosed along with the application. The envelope containing the above documents should be superscribed "Suman Food Consultants Travel Award – 1988".

BEST STUDENT AWARD

The award is to be given to a student having a distinguished academic record and undergoing the final year course in Food Science and Technology in any recognised University in India. The aim of the award is to recognise the best talent in the field and to encourage excellence amongst the student community.

The guidelines for the Award are:

- (i) The applicant must be an Indian national
- (ii) He/she must be a student of one of the following courses:
 - (a) M.Sc. (Food Science)/(Food Technology)
 - (b) B.Tech., B.Sc. (Tech), B.Sc. (Chem. Tech) with Food Technology specialisation.
- (iii) He/she should not have completed 25 years of age on 31st December 1988.

Heads of the Department of Food Science and Technology in various Universities may sponsor the name of one student from each institution supported by the candidate's biodata, details starting from high school onwards, including date of birth and post-graduate performance to date (five copies).

The envelope containing the nomination should be superscribed "Nomination for Best Student Award – 1988".

YOUNG SCIENTISTS AWARD

This award is aimed at stimulating distinguished scientific and technological research in the field of Food Science and Technology amongst young scientists in their early life.

The guidelines for the Award are:

1. The candidate should be an Indian national below the age of 35 years on 31st December 1988, working in the area of Food Science and Technology.
 - (i) The Candidate should furnish evidence of either:
 - (a) Original scientific research of high quality, primarily by way of published research papers and (especially if the papers are under joint authorship) the candidate's own contribution to the work.

OR

- (b) Technological contributions of a high order, as reflected by accomplishments in process design etc., substantiated with documentary evidence.

The application along with details of contributions of biodata (five copies) may be sent by registered post with the envelope superscribed: "Nomination for Young Scientists Award 1988"..

ASSOCIATION OF FOOD SCIENTISTS & TECHNOLOGISTS (INDIA)

CFTRI Campus, Mysore-570 013, India

NOMINATIONS FOR AFST (I) AWARDS FOR 1988

Nominations for the following awards of the AFST (I) for the year 1988 are invited. All nominations should be sent by Registered post, so as to reach Honorary Executive Secretary, Association of Food Scientists and Technologists (India), CFTRI Campus, Mysore-570 013, before 31 October 1988.

PROF. V. SUBRAHMANYAN INDUSTRIAL ACHIEVEMENT AWARD

The guidelines for the award are:

- (i) Only Indian nationals with outstanding achievement in the field of Food Science and Technology will be considered for the award.
- (ii) The nominee should have contributed significantly to the enrichment of Food Science and Technology, and the development of agro-based food and allied industries in India.
- (iii) The nomination duly proposed by a member of the Association must be accompanied by the biodata of the candidate highlighting the work done by him for which he is to be considered for the award.
- (iv) The awardee will be selected by an expert panel constituted by the Central Executive Committee of the Association.

The envelope containing the nominations along with biodata and contributions (five copies) should be superscribed "Nomination for Prof. V. Subrahmanyam Industrial Achievement Award – 1988".

LALJEE GODHOO SMARAK NIDHI AWARD

The guidelines for the award are:

- (i) The R & D group/person eligible for the award should have contributed significantly in the area of Food Science and Technology in recent years with a good standing in his/her field of specialisation.
- (ii) The nominee(s) should be duly sponsored by the Head of the respective Scientific Institution and the application for this award should highlight complete details of the contributions made by the candidates and their significance.
- (iii) The awardee(s) will be selected by an expert panel constituted by the Central Executive Committee of the Association.

The envelope containing the nominations (five copies) should be superscribed "Nomination for Laljee Godhoo Smarak Nidhi Award 1988".

INSTRUCTIONS TO AUTHORS

1. Manuscripts of papers (in triplicate) should be typewritten in double space on one side of bond paper. They should be complete and in final form, since only minor corrections are allowed at the proof stage. The submitted paper should not have been published or data communicated for publication anywhere else. Only invited review papers will be published.
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. Methods of sampling, number of replications and relevant statistical analyses should be indicated. Superscripts and subscripts should be legibly and carefully placed. Foot notes especially for text should be avoided as far as possible.
4. **Abstract:** The abstract should indicate the principal findings of the paper. It should be about 200 words and in such a form that abstracting periodicals can readily use it.
5. **Tables:** Tables as well as graphs, 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. They should be typed on separate sheets. Nil results should be indicated and distinguished clearly from absence of data, which is indicated by '—' sign. Tables should not have more than nine columns.
6. **Illustrations:** Graphs and other line drawings should be drawn in *Indian ink* on tracing paper or white drawing paper preferably art paper not bigger than 20 cm (oy axis) × 16 cm (ox axis). The lettering should be such that they are legible after reduction to column width. Photographs must be on glossy paper and must have good contrast; *three 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 along with title of the paper should be cited completely in each reference. Abbreviations such as *et al.*, *ibid*, *idem* should be avoided.

The list of references should be included at the end of the article in serial order and the respective serial number should be indicated in the text as superscript.

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

- (a) *Research Paper:* Jadhav, S. S. and Kulkarni, P. R., Presser amines in foods. *J. Fd Sci Technol.*, 1981, **18**, 156.
 - (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 Papers:* Nambudiri, E. S. and Lewis, Y. S., Cocoa in confectionery, *Proceedings of the Symposium on the Status and Prospects of the Confectionery Industry in India*, Mysore, May 1979, 27.
 - (e) *Thesis:* Sathyanarayan, Y., *Phytosociological Studies on the Caliculous Plants of Bombay*, 1953, Ph.D. Thesis, Bombay University.
 - (f) *Unpublished Work:* Rao, G., unpublished, Central Food Technological Research Institute, Mysore, India.
9. Consult the latest issue of the *Journal* for guidance and for "Additional Instructions for Reporting Results of Sensory Analysis" see issue No. 1 of the Journal.

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