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ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS

(INDIA)

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OBITUARY

DR. P. B. RAMA RAO

Dr. P. B. Rama Rao, a former Editor of the Journal of Food Science and Technology passed away suddenly on 13th August 1982 at the prime age of 52 due to heart attack.

Dr. Rama Rao was born on 10th August 1930, and had his education in Bangalore. He took B.Sc (Hons.) degree in Chemistry from the Central College, Bangalore. In 1950 he joined the Indian Institute of Science, Bangalore and worked on nutrition under the guidance of Dr. R. Rajagopalan and Prof. K. V. Giri. This work was awarded the Ph.D. degree by Bombay University in 1956.

He went to the University of Illinois in 1955 on a J. N. Tata Endowment Loan Scholarship, and worked with Prof. B. Connor Johnson for five years in the division of animal nutrition. His work dealt with the essential aminoacid requirements of the growing rat. He also studied vitamin K deficiency caused by feeding irradiated meat, and the involvement of vitamin K in oxidative phosphorylation. Later, at the Karolinska Institute, Stockholm, Sweden, he was involved in clinical research on folate metabolism in neoplastic states. Many years later, he worked for a short time in West Germany on a DAAD fellowship.

After a brief period as a CSIR Pool Officer at the Indian Institute of Science, Bangalore, he joined the Central Food Technological Research Institute in 1964. Dr. Rama Rao and his group investigated various aspects of clinical and experimental vitamin A defficiency, specially mucopolysaccharide metabolism, and the effect of Vitamin A on (*i*) red cell membrane proteins, (*ii*) differentiation of intestinal cells, (*iii*) process of myelination in the brain, and (*iv*) immunological status. He built one of the most active research groups working on vitamin A in India. He also initiated some work on the fatty acid profile of tissue lipids in vitamin B_{12} defficiency.

As a research worker, Dr. Rama Rao was full of novel ideas and never chose to work on "run of the mill" problems. He also contributed substantially to the scientific life of the community by playing several active roles in the Society of Biological Chemists of India, of which he was the Secretary and President of Mysore Chapter, and the Association of Food Scientists and Technologists (India), of which he was the Secretary and Vice-President. Moreover, he edited the Journal of Food Science and Technology from 1974 to 1976. He was also Vice-President of the CFTRI Education Society and CFTRI Scientific Workers' Association for some time.

As a scientist, Dr. Rama Rao was an embodiment of some of the finest traditions and virtues that are associated with the pursuit of science. He combined a keen and analytical mind with honesty of purpose, and complete objectivity in establishing facts and arriving at a judgement. His affable and open-hearted nature won him many life-long friends who were shocked at the news of his sudden death. Research fellows working for doctorates regarded him with great love and reverence.

Unassuming, modest, possessing an unquestionable competence and the willingness to persevere at any long and difficult task, Dr. Rama Rao was a product of that generation which grew up in the princely state of Mysore just before Independence. One can only hope and pray that an equally able new generation will take his place, and try to emulate him in his dedication to constructive endeavour.

D. Rajagopala Rao
K. M. Dastur
Central Food Technological Research Institute, Mysore-570 013.

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JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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RESEARCH PAPERS

Studies on the Production of ∝-Amylase by *Bacillus subtilis* Growing in the Batch and Chemostat Cultures*

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Manuscript received 29 *January* 1981

Production of ∝-amylase by *Bacillus subtilis* NCIB 8646 was investigated in batch and continuous cultures on a synthetic medium. In batch cultures, the rate of ∝-amylase synthesis reached the maximum in postlogarithemic phase. The exogenous additions of glucose and acetic acid to control the pH increased significantly the cell yields and the enzyme forming system. In chemostat cultures, the maximum synthesis of ∝-amylase was associated with carbon limited growth rate at a dilution rate of 0.2/hr^{-1}. ∝-amylase was also induced in the other conditions namely, nutrient limitations other than the carbon limitation. Higher concentrations of glucose in the medium severely repressed the enzyme forming system. It supports the earlier views on the catabolic repression of this enzyme system. Glucose and soluble starch induced a higher enzyme forming system. Organic nitrogen sources yielded more active amylase than the inorganic nitrogen sources.

Alpha amylase (∝ 1, 4 glucan-4-glucano hydrolase (E.C. 3.2.1.1) produced by *Bacillus subtilis* has been extensively used in various industries. In recent years a number of publications have appeared on the synthesis of ∝-amylase using very complex media. Very little information is available regarding the production of ∝-amylase on synthetic media. In order to have a better understanding of the effect of various physiological conditions on the enzyme forming system, the author tried to use a synthetic medium. The present investigation has been directed towards optimising the production of ∝-amylase by *Bacillus subtilis* NCIB 8646 growing in batch and chemostat cultures.

Materials and Methods

Organism and its maintenance: *Bacillus subtilis* NCIB 8646 was used in the present investigation. It was maintained on nutrient agar slopes by subculturing at monthly intervals.

Medium composition: A chemically well defined medium[1] (containing glucose (10g/l))as the principal carbon source was used in most of the experiments.

Batch cultures: The bacteria were grown in a 5 l. fermentor with a normal working volume of 3 l. The air flow and agitation rates were 1500 ml/min and 700 rev/min respectively. The pH of the culture was maintained constant at 7.0±0.1 via an automatic magnetic valve system, which regulated the supply of either 2N NaOH or 2N H_2SO_4 unless otherwise stated. The temperature was thermostatically maintained at 37± 0.5°C. The foam was controlled with 0.05 per cent pluronic L. 61.

Chemostat cultures: The bacteria were grown in 1 l. (Port—on type) chemostat with 0.54 l. working volume. The agitation and air flow rates were 1000 rev/min and 540 ml/min respectively. The flow rate of the medium was regulated at desired dilution rates with a Watson-Marlow MHRE-7 pump (Watson-Marlow Ltd., Falmonth, Cornwall, England). Other conditions were same as in batch cultures.

Preparation of inoculum: For inoculating the fermentors a liquid inoculum was prepared by suspending a loopful of spores from the freshly cultured nutrient agar slopes in 100 ml sterile medium contained in a 500 ml baffled flask. Incubation was made at 37°C on a rotary shaker at 200 rev/min for 24 hr.

Measurement of growth: Bacterial growth was followed by measuring the OD at 525 nm in a spectronic 20. A portion of the suspension (10 ml) was centrifuged in pre-weighed glass tubes at 3000 rev/min for 15 min and the pellet was washed with distilled water and dried at 105°C for 24 hr and weighed (X).

Determination of ∝-amylase activity (E): The activity was estimated by using the NOVO-method (NOVO Industry, DK 2880—Bagsvaerd, Denmark) using starch and iodine. An enzyme unit (NU) is defined as that

*A part of the post doctoral research carried out by the author at the Department of Applied Bio Chemistry. The Technical University of Denmark, Copenhagen, Denmark.

amount of enzyme in one hour under standard conditions (37°C, 0.0043 M Ca^{++}, pH 5.6–5.7) breaks 5.26 mg starch to a certain iodine colour measured by a Hellige NEO comperator (Hubert Lanch Jr. Inc., Westburry, New York).

Analysis of growth rate and specific productivity: From the measured values of X and E the specific growth rate $\mu = \frac{1}{x} \cdot \frac{dx}{dt}$ and the specific productivity $\epsilon = \frac{1}{x} \cdot \frac{dE}{dt}$ were calculated using a desk computer. The plots for X and E versus the time 't' was assumed to be linear for small intervals (1 hr).

Results and Discussion

Time course of a normal batch culture: The progress of the bacterial growth and ∝-amylase production are shown in Fig. 1(*a*). The pH was maintained at 7.0 with 2N H_2SO_4. The maximum cell concentration was 1.8 g/l and the maximum level of enzyme concentration was 192 KNU/l. In Fig 1(*b*), the corresponding values of μ and ϵ are plotted. The maximum growth rate was 0.36 h^{-1} with a maximum specific productivity of 18 KNU/g/hr. A low differential rate of ∝-amylase secretion was observed during logarithemic phase of growth which undergoes a dramatic increase in the stationary phase. The observation was in accordance with the earlier workers[2-4].

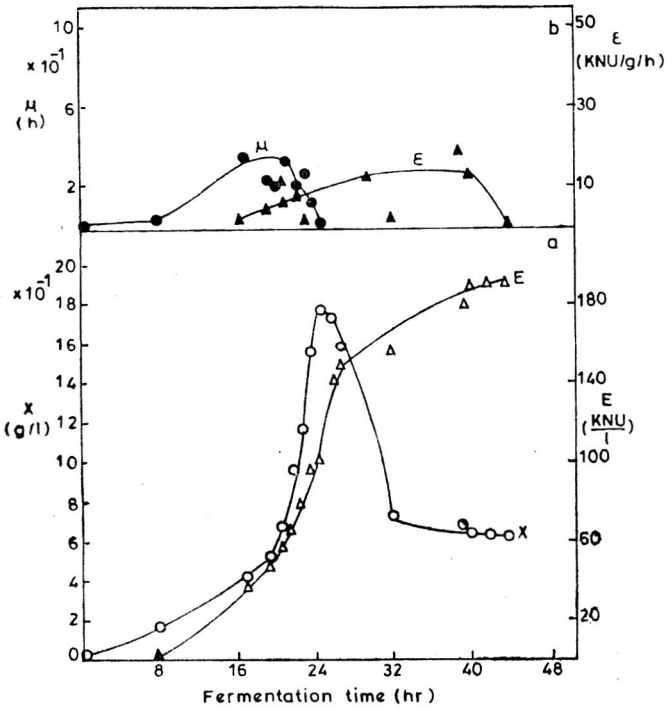

Fig. 1. Time course of a normal batch culture.

Effect of maintaining pH with 50 per cent glucose and 2 N acetic acid: When the glucose in the medium was nearly exhausted the pH of the culture broth turns towards alkaline range due to the formation of certain basic metabolites. It was normally possible to control the pH at this stage with 2N H_2SO_4. In the present investigation, efforts were made to study the influence of added glucose or acetic acid to the cultures, when the original glucose in the media was almost consumed. The results are given in Fig 2(a & b) and 3 (a & b). In both the cases the cell growth was typically diauxic. When 200 ml of 50 per cent glucose was added the maximum cell concentration was increased from 2.4 to 7.2 g/l and the enzyme activity was also increased to 280 KNU/l (Fig. 2*a*). The maximum specific growth rate in the early phase of the growth was 0.4 hr^{-1}. The total concentration of the enzyme was increased over the control (Fig. 1) and the maximum specific productivity was 12 KNU/g/hr (Fig. 2*b*). In another experiment when 175 ml of 2N acetic acid was added, the cell yields were increased from 1.75 to 5.2 g/l with a maximum enzyme activity of 384 KNU/l (Fig. 3*a*). The maximum specific growth rates were 0.39 and 0.2 h^{-1} in the former and latter growth phases respectively. The specific productivity even in this was 5 KNU/g/hr (Fig. 3*b*). Thus when the added carbon sources were glucose or acetic acid, a greater deviation in the ∝-amylase production from the

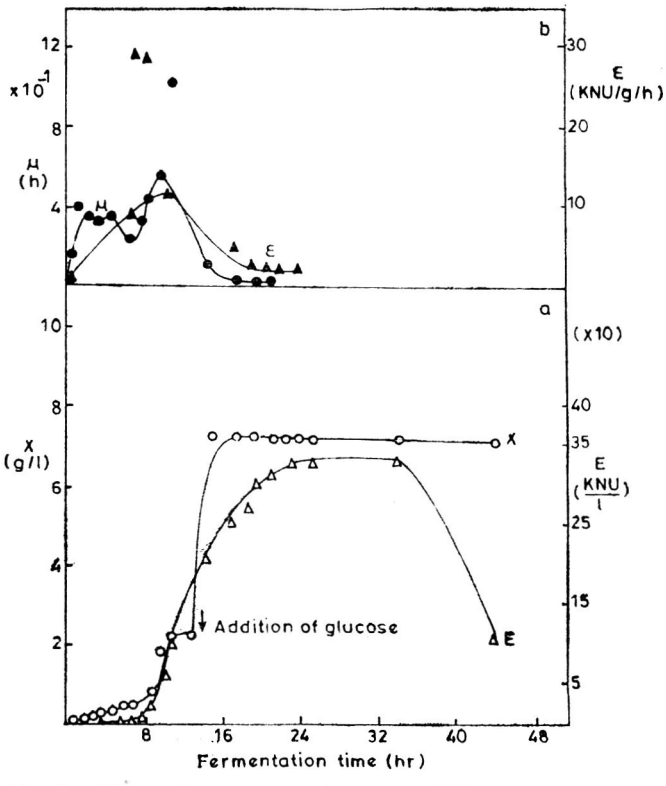

Fig. 2. Effect of exogenous addition of 50% glucose on ∝-amylase production.

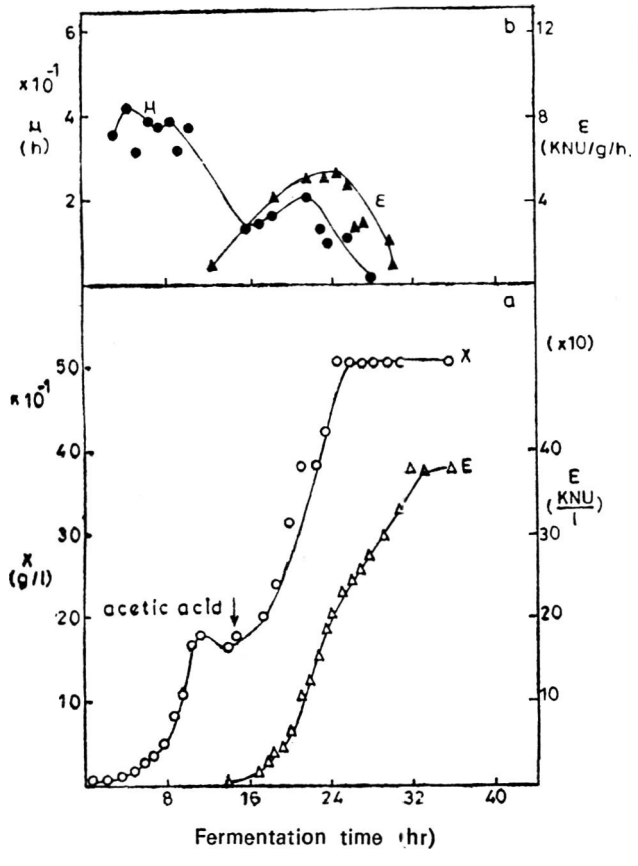

Fig. 3. Effect of exogenous addition of 2N acetic acid on the formation of ∝-amylase.

control was observed. But this is accountable with the accompanying significant increase in the biomass in the presence of these carbon sources.

Single stage continuous cultures: Continuous cultures studies were made to have a better understanding of the regulatory mechanisms involved in the biosynthesis of ∝-amylase. In the following experiments, the behaviour of ∝-amylase production in continuous culture was examined.

Dependance of ∝-amylase synthesis on dilution rate: The bacteria were grown in glucose limiting, mineral salts medium. Over a range of dilution rates from 0.05 to 0.5 hr^{-1} (Fig. 4), dilution of 0.2 hr^{-1} was found to be the optimum for the elaboration of maximum ∝-amylase (244 KNU/l). At dilution rates exceeding this value strongly repress the ∝-amylase production and at dilution of 0.5 hr^{-1}, a complete wash out of the cells was observed.

Effect of carbon sources: The effect of carbon sources on ∝-amylase production was determined by incorporating various carbohydrates (1 per cent w/v). When the organism was grown on different carbon sources as shown in Fig. 5, it exhibited different specific growth rates (unpublished data). But in the present study comparison was made within the carbon sources at dilution

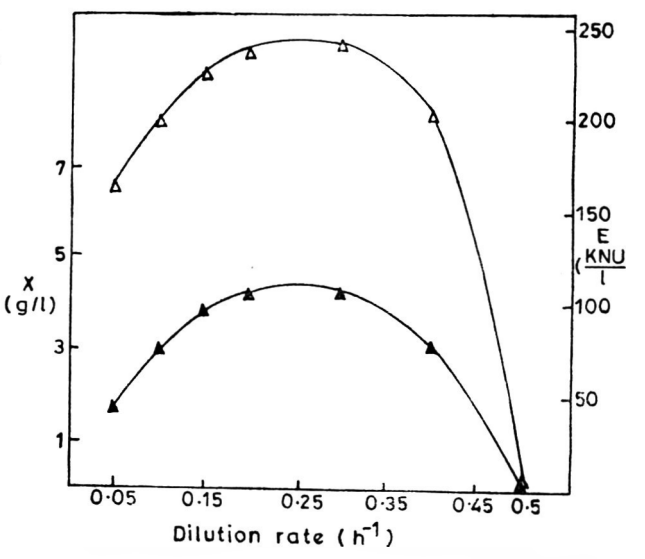

Fig. 4. ∝-amylase production as a function of dilution rate.

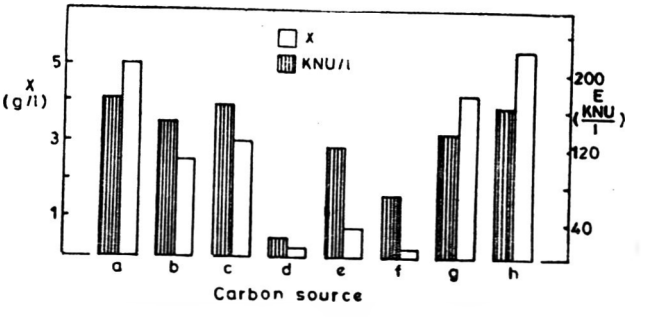

Fig. 5. The relation between ∝-amylase production (E) and different carbon limitations.

a) glucose; *b*) maltose; c) sucrose; *d*) lactose; *e*) glycerol; *f*) galactose; *g*) dextrin; *h*) starch.

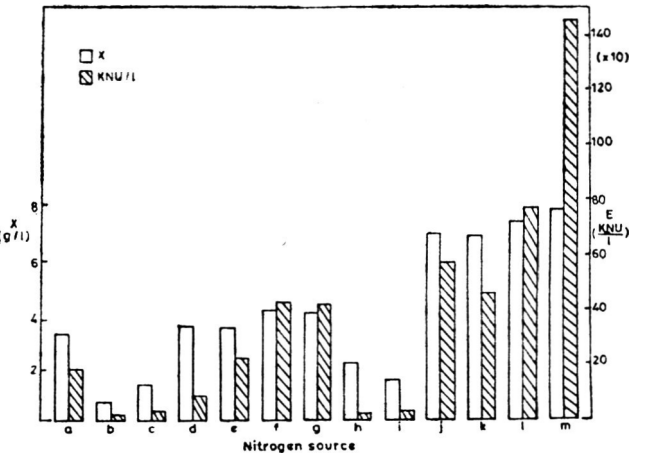

Fig. 6. Effect of different nitrogen sources on the production of ∝-amylase.

a) Ammonium chloride; *b*) Sodium nitrite; *c*) Ammonium carbonate, *d*) Potassium nitrate; *e*) Ammonium hydrophosphate; *f*) Ammonium sulphate; *g*) Ammonium nitrate; *h*) Urea; *i*) Beef extract; *j*) Casein; *k*) Yeast extract; *l*) Tryptone, *m*) Peptone.

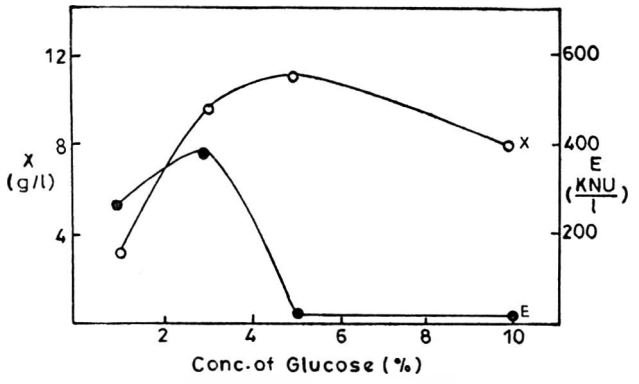

Fig. 7. Effect of different concentrations of glucose on ∝-amylase production.

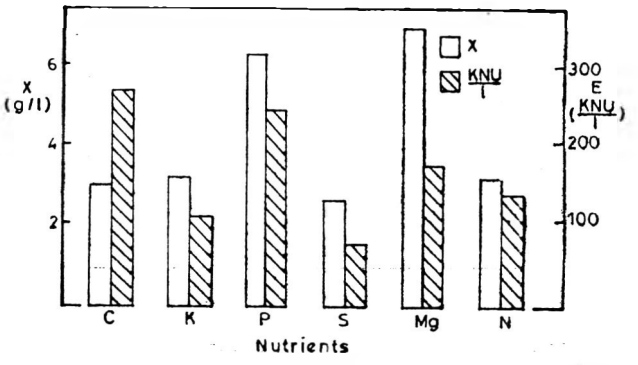

Fig. 8. Production of ∝-amylase (E) in different nutrient limitations.

The limiting concentrations are 1% glucose, 2M phosphate, 1mM potassium, 0.4 mM sulphur, 2.5 mM magnesium, 30 mM nitrogen.

of 0.2 hr^{-1}, as it was found optimum in most of the cases. Glucose and soluble starch induced a higher enzyme forming system while the other sugars yielded very little or no ∝-amylase formation. It supports the results obtained earlier.[5]

Effect of nitrogen sources: In examining the effect of nitrogen sources, ammonium chloride in the original mineral salts medium was replaced by equimolar concentrations of inorganic nitrogen source or 1 per cent (w/v) of organic sources. The results are shown in Fig. 6. In general the organic nitrogen sources (1 per cent w/v) gave more active amylase than the inorganic nitrogen sources. Highest activity was observed with peptone (1440 KNU/l). These results are in accordance with the observations of Dias and Henkelekian[6].

Effect of glucose content: Different levels of glucose were used to find out the optimum concentration for the synthesis of ∝-amylase at dilution 0.2 hr^{-1}. The results are presented in Fig. 7. Glucose at 3 per cent concentration resulted in maximum enzyme production (396 KNU/l). However, at higher concentration, lower yield was obtained. Similar results were obtained in a variety of organisms[7]. The inhibitory effect was not due to the oxygen limitation. It was found that even at this critical stage, the dissolved oxygen is about 50 per cent saturation, which most probably maintains the aerobic conditions. Mandelstem[8] postulates the concept of a catabolite repressing only the enzyme directly or indirectly producing them. The catabolite repression is conspicuous generally by the presence of higher concentrations of rapidly metabolised carbon sources. Thus the observed lower activities at higher concentrations of glucose is attributed to its catabolic repression on the enzyme forming system.

Effect of nutrient limitations: Experiments were conducted with various nutrient limitations in the growth medium in order to find out their influence on the production process. The results are presented in Fig. 8. Carbon and phosphorus limitations favour the higher production of ∝-amylase. The ∝-amylase activity with the medium containing 1 per cent glucose as a limiting substrate was 244 KNU/l while with phosphorus it was 212 KNU/l. Heineken and O'Connor[9] also observed that the limiting growth with glucose was advantageous for ∝-amylase production in *Bacillus subtilis*. Markkanen and Magnus[10] reported the stimulation of ∝-amylase production by *Bacillus subtilis* when grown in a phosphate limiting medium.

Acknowledgement

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Enzymatic Browning in Some Banana Varieties as Related to Polyphenoloxidase Activity and Other Endogenous Factors

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Polyphenoloxidase (PPO) activity and level, ascorbic acid, total phenolic content and pH were determined and compared in the ripe pulp of five major banana cultivars and correlated with the differences in their rate of enzymatic browning. Browning in bananas was influenced mainly by PPO activity and level, and ascorbic acid content which showed differences among the cultivars. Varieties relatively low in PPO activity and concentration, and high in ascorbic acid exhibited the least browning. The natural pH of the pulp was also found to exert some influence on browning, nearer the pH to 7.0, the higher the susceptibility.

No significant differences were observed in the total phenolic content of the five varieties and this did not therefore, appear to be a factor limiting browning in bananas. Experiments also ruled out possible involvement of endogenous PPO inhibitors in low active varieties and activators in high active ones. PPO activity and concentration were almost twice during winter months as compared to those during summer suggesting that seasonal variation could be utilised to advantage in selecting raw material with the least PPO activity to minimise browning during processing.

Banana is an important world fruit crop and represents about 40 per cent, by weight, world trade in fruits, fresh or dried[1]. The fruit contains polyphenoloxidase (PPO) enzyme system and is therefore, susceptible to browning when cut or injured. Palmer[2] isolated the enzyme from the pulp and found dopamine to be its most reactive substrate while Griffiths[3] confirmed the presence of dopamine and demonstrated it to be the primary substrate in browning.

It is now well recognised that the factors responsible for enzymatic browning are influenced by varietal characteristics and to minimise browning during processing, it is desirable and useful to select the least susceptible varieties. Numerous varieties of banana of commercial importance are cultivated in abundance in various parts of India[4]. The fruit has been processed into several products, canned and dehydrated. No attempt has, however, so far been made to compare different varieties with respect to their susceptibility to enzymatic browning and to correlate the same with the PPO level and other endogenous factors influencing browning, with a view to identify varieties least susceptible to browning.

In the present study, PPO activity and its level, total phenolic compounds, ascorbic acid and pH were determined in the pulp of five commercially important varieties of ripe banana and correlated with the differences in their susceptibility to browning. Possible influence of endogenous inhibitors or activators on PPO activity and variation of PPO activity and concentration with season were also investigated.

Materials and Methods

Banana pulp tissue from the following five commercially important varieties[4,5] of ripe fruits (skin colour: yellow to yellow with brown flecks) available in the local market was used. 'Pachabale' or 'Dwarf cavendish' (*M. cavendishii*; AAA); 'Poojabale' or 'Poovan' (*M. paradisiaca*; AAB); 'Rasabale' or 'Rasthali' (*M. sapidisiaca*; AAB); 'Kadubale' (*M. paradisiaca*; AAB) and 'Puttabale' (*M. paradisiaca*; AB).

Soluble and total solids: The soluble solids content of the pulp was measured directly in °Brix at room temperature (25-30°C) and the total solids by drying in a vacuum oven.

Titratable acidity and pH: Acidity was determined by titrating the pulp extract with 0.1N NaOH using phenolphthalein as indicator and expressed as per cent anhydrous citric acid. The pH of the blended pulp was measured using a standard digital pH meter.

Ascorbic acid: Ascorbic acid was extracted and assayed by the 2,6-dichlorophenol indophenol titrimetric method[6] and expressed as mg per 100 g pulp.

Total phenolics: Total phenolic content was estimated colorimetrically by a method similar to that described by Guadagni *et al.*[7] using Folin Denis reagent and expressed as mg anhydrous tannic acid per g pulp.

Polyphenoloxidase: PPO was extracted and assayed by the method of Palmer[2]. Extraction was done using 0.1 M potassium phosphate buffer pH 7.0 containing a nonionic detergent (Tween 80) at 1 per cent level. Incorporation of a phenolic binder, polyvinylpyrrolidone (PVP), in the extracting buffer upto 5 per cent level did not result in any increase in PPO activity and so it was not used.

Activity was assayed by measuring the rate of increase in absorbance at 475 nm at 25°C in a Perkin-Elmer model 124 double-beam grating spectrophotometer or a Bausch and Lomb spectronic 20 colorimeter using dopamine as substrate. The increase in absorbance which occurred within the first 2 min of the reaction time was taken as rate of reaction. One unit of PPO activity was defined as the amount of enzyme that caused a unit change in absorbance per minute at 475 nm.

Protein content was estimated in the enzyme extracts after suitable dilution with water by the method of Lowry *et al.*[8] using bovine serum albumin as standard. Specific activity of the enzyme was expressed as units per mg protein. PPO concentration was also calculated where necessary, as activity units per gram fresh pulp.

Optimum pH for banana PPO activity: In studies on determination of pH optimum for banana PPO activity, activity of the crude enzyme was measured as above using appropriate 0.1 M buffers in the pH range of 3.0 10.0 to (pH 3 to 6, citrate buffer; pH 6-8, phosphate buffer; pH 8-9, Tris-HCl buffer and pH 9-10 carbonate buffer). Activity was plotted against pH to obtain the pH optimum.

Susceptibility to enzymatic browning: Susceptibility of the pulp tissue to enzymatic browning was determined by visual observation as well as by reflectance measurements. The peeled fruit was cut longitudinally into half and the cut surface kept exposed to air at room temperature. Extent of browning was recorded visually at intervals of 30 min upto 6 hr.

For reflectance measurements, about 50 g pulp was ground in a glass pestle and mortar and quickly transferred to a sample holder (opaque plastic dish of 1 inch sq). Per cent diffuse reflectance was measured initially and after every 10 min upto 60 min and then every 1 hr upto 6 hr with white light using a reflectance meter (AIMIL). Magnesium oxide was used as standard to set the instrument to 100 per cent reflectance. Per cent reflectance was plotted against time in a graph and the drop in reflectance ($\triangle$R) occurring in 60 min was used as a measure of the browning potential.

Results

PPO activity and other endogenous factors in banana varieties: Some physico-chemical characteristics of the pulp of five varieties of ripe banana studied are given in

TABLE 1. PHYSICO-CHEMICAL CHARACTERISTICS OF SOME VARIETIES OF RIPE BANANA

Characteristics	Pachabale	Poojabale	Rasabale	Kadubale	Puttabale
°Brix (at 25°C)	20.00	23.50	27.50	24.00	30.00
Total solids (%)	22.35	28.08	32.48	29.72	37.00
pH	5.15	4.45	4.60	4.50	4.60
Total acidity (as anhyd citric acid %)	0.25	0.41	0.32	0.25	0.33
Brix/Acid ratio	80	56	86	96	91
Ascorbic acid* (mg/100g)	1.22 $\pm$ 0.21	1.77 $\pm$ 0.17	9.30 $\pm$ 0.87	1.67 $\pm$ 0.06	7.14 $\pm$ 0.77
Total phenolic compounds* (as tannic acid, mg/100g)	55.33 $\pm$ 12.31	44.03 $\pm$ 4.90	49.67 $\pm$ 5.43	44.60 $\pm$ 2.30	42.60 $\pm$ 4.45
Initial brownness (% reflectance)	54	45	44	50	50
Drop in % reflectance in 1 hr ($\triangle$ R)	29	18	9	12	8

*Figures (Mean $\pm$ S.D.) are from six replicates of each variety drawn from different lots. All other values are average of minimum three determinations.

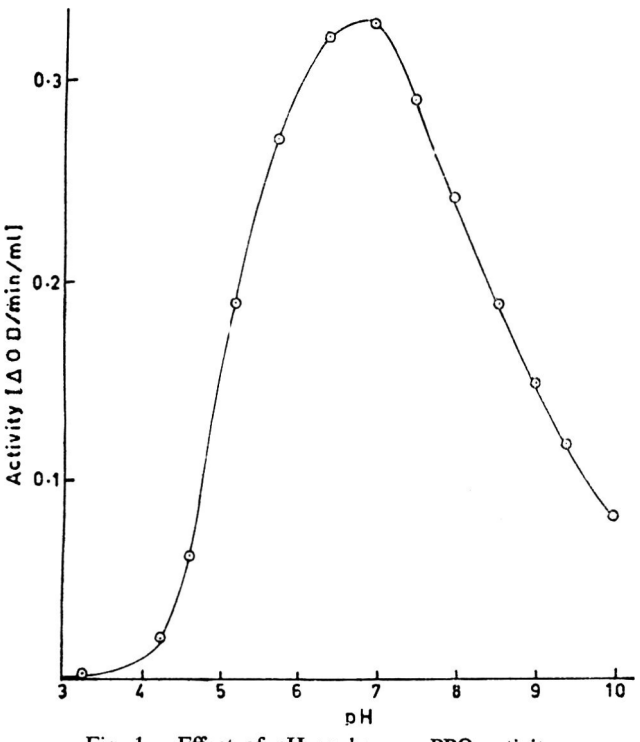

Fig. 1. Effect of pH on banana PPO activity

Table 1. Soluble solids and total solids content showed differences among the varieties with 'Pachabale' having the lowest and 'Puttabale' the highest with others ranging in between. 'Poojabale' had the highest titratable acidity and the lowest Brix/acid ratio as compared to others indicating it to be sourer than others.

It was interesting to note that the pH of 'Pachabale' was significantly high (5.2) as compared to the value of 4.5 to 4.6 associated with the others. The optimum pH for banana PPO activity was 7.0 as judged by measurements using the crude enzyme extract (Fig. 1). At pH 5.2, the PPO of 'Pachabale' exhibited 57.5 per cent of its activity on comparison with pH 7.0 value. At the pH of 4.5 to 4.6 associated with the other varieties, it showed only 6-11 per cent of its activity at the optimal pH.

Ascorbic acid was higher (about five times) in 'Rasabale' and 'Puttabale' compared to the low values observed in other three varieties. The five varieties did not show any significant differences in their total polyphenol content when estimated as tannic acid.

PPO activity, specific activity and concentration per gram of fresh tissue were relatively highest in 'Pachabale' and lowest in 'Kadubale' and 'Puttabale' (Table 2). The values were in the order: 'Pachabale'> 'Poojabale' > 'Rasabale' > 'Kadubale' = 'Puttabale' While 'Rasabale' showed intermediate values, those of 'Kadubale' and 'Puttabale' were significantly low, almost one sixth of 'Pachabale', one fourth of 'Poojabale' and half of 'Rasabale'.

Susceptibility to browning: Susceptibility to browning upon cutting as judged by visual observation showed that in 'Pachabale' and 'Poojabale' varieties there was significant browning around the core within 30 min, spreading towards periphery in 4 hr. Fifty per cent of of the pulp around the core became brown in 4 hr while the peripheral pulp was slightly brownish. Core finally turned into brownish black in 6 hr. 'Rasabale' and 'Puttabale' showed no signs of browning in 6 hr with their flesh remaining white. 'Kadubale' exhibited slight browning as compared to 'Rasabale' and 'Puttabale' but considerably less than 'Pachabale' and 'Poojabale'.

Reflectance measurements have been used to measure enzymatic browning in freshly cut or injured fruit tissues. Thus decrease in reflectance of injured fruit at 545 nm was used as a measure of browning phenomenon in green olives[9], while change in lightness obtained from measurements of reflectance initially and after 3 hr exposure to air at room temperature was used for quantitative evaluation of browning potential in avocado[10].

TABLE 2. POLYPHENOL OXIDASE ACTIVITY AND PROTEIN[a] IN FRESH HOMOGENATES[b] OF SOME VARIETIES OF RIPE BANANA

Variety	PPO. activity (units/ml)	Protein (mg/ml)	Specific activity (units/mg protein)	PPO. concn (units/g pulp)
Pachabale	6.39±1.33	1.34±0.10	4.83±0.98	63.92±13.32
Poojabale	3.87±0.52	1.24±0.11	3.12±0.56	38.75± 5.18
Rasabale	2.67±0.26	1.30±0.15	2.04±0.40	26.66± 2.58
Kadubale	1.19±0.24	1.51±0.24	0.87±0.22	11.9 ± 2.46
Puttabale	1.17±0.33	1.50±0.34	0.78±0.09	11.70± 3.35

[a]Figures are from six replicates of each variety drawn from different lots.

[b] 2g pulp homogenised with 20 ml of a 1% detergent solution (Tween 80) buffered at pH 7.0 with 0.1M potassium phosphate; homogenate centrifuged at 20,000xg for 15 min at 0°C and supernatant used for assay.

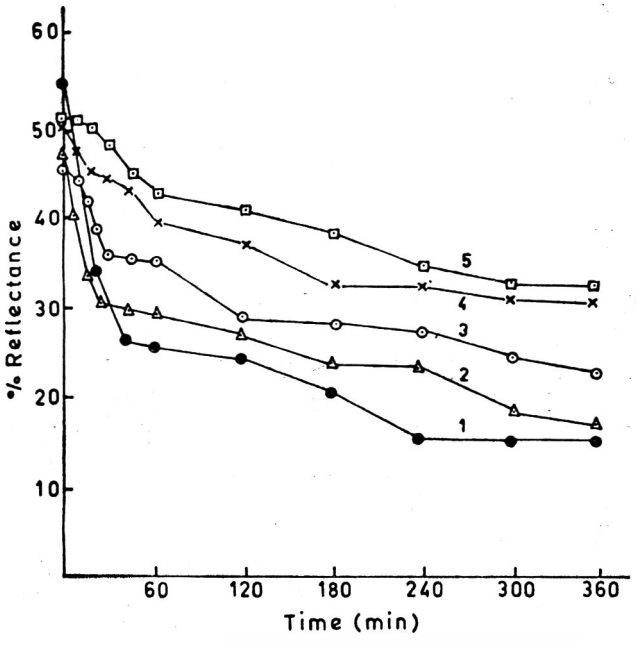

Fig. 2. Reflectance as ameasure of anezymtic browning in banana varieties.
1. Pachabale 2. Poojabale 3. Rasabale 4. Kadubale 5. Puttable.

In the present studies on browning in bananas the visual observations were generally in agreement with the observations made by measuring the per cent reflectance of the ground pulp over a period of 6 hr (Fig. 2). As seen from the graph and as judged by the drop in per cent reflectance from the initial value to the values after 60 min ($\triangle$R), susceptibility to browning in the five varieties was found to be in the order: 'Pachabale'> 'Poojabale'>'Kadubale'>'Rasabale'='Puttabale'.

Influence of endogenous inhibitors or activators on PPO activity in banana varieties: The possiblity that the relatively low PPO activity exhibited by 'Rasabale', 'Kadubale' and 'Puttabale' was due to the presence of an inhibitor or to factors degrading the enzyme such as endogenous proteolytic activity and the high PPO activity of 'Pachabale' was due to an activator was investigated and ruled out by methods similar to those used by Kahn for avocado PPO[11].

The pulp from each of the three low active varieties was mixed individually with equal amount of 'Pachabale' pulp and the enzyme extracted and assayed immediately. The observed activity was very close to that expected from computation of the activity obtained when the corresponding amounts of each variety were extracted and assayed separately.

The crude enzyme from each of the three low active varieties was mixed individually with crude 'Pachabale' enzyme and the mixture assayed immediately. The observed activity was again found to be close to that expected from computation of the activities obtained when the corresponding amounts of each enzyme were assayed separately.

Addition of crude enzyme from the three low active varieties after inactivation by boiling (which is likely to liberate an inhibitor from the enzyme-inhibitor complex) individually to the fresh crude 'Pachabale' enzyme had no effect on the activity of the latter. Extensive dialysis of the crude enzyme extracts from the low and high active varieties (which is likely to remove an inhibitor or activator molecule) also showed no effect on the relative differences in their activities and specific activities.

Seasonal variation in PPO activity and concentration: Specific activity and concentration of PPO were determined in the crude extract from four banana varieties at various times during the year to find out any seasonal variation. As seen from the data in Table 3, a sharp

TABLE 3. SEASONAL VARIATION IN THE ACTIVITY, SPECIFIC ACTIVITY AND CONCENTRATION OF PPO (CRUDE EXTRACT*) FROM SOME VARIETIES OF RIPE BANANA

Variety	Month	PPO. activity (units/ml)	Protein (mg/ml)	Specific activity (units/mg protein)	PPO. concn. (units/g pulp)
Pachabale	Jan	8.0	1.30	6.1	80
	Feb	6.1	1.43	4.3	61
	Mar	5.0	1.51	3.3	50
	May	5.0	1.42	3.5	50
	Jul	5.0	1.20	4.2	50
	Sep	8.5	1.27	6.7	85
	Oct	10.5	1.34	7.8	105
	Nov	10.0	1.53	6.5	100
Poojabale	Jan	3.75	1.30	2.9	37.5
	Apr	3.25	1.40	2.3	32.5
	Jul	4.50	1.10	4.1	45.0
	Aug	3.25	1.12	2.9	32.5
	Nov	7.90	1.25	6.1	79.0
	Dec	4.00	1.30	3.1	40.0
Rasabale	Feb	2.45	1.08	2.3	24.5
	Apr	2.60	1.22	2.1	26.0
	Aug	2.80	1.15	2.4	28.0
	Dec	7.25	1.12	6.5	72.5
Kadubale	Jan	1.16	1.24	0.9	11.6
	Feb	1.20	1.28	0.9	12.0
	Mar	0.90	1.60	0.6	9.0
	Apr	0.60	1.80	0.3	6.0
	Dec	1.50	1.40	1.1	15.0

*2 g pulp extracted with 20 ml 1% detergent buffer pH 7.0 and centrifuged.

increase in PPO activity was observed during the winter months (November-December) to almost double the value found during the summer months (April-August) in all the varieties. The protein content did not, however, show any similar increase. The specific activity of the crude enzyme extract was, therefore, the highest in winter.

Discussion

Although enzymatic browning of fruits is mainly attributed to the action of PPO, knowledge accumulated on this subject is divergent and not clear as to whether the enzyme or the substrate plays the decisive role in the overall phenomenon of browning. Thus, degree of browning was found to correlate with PPO activity in peaches[12], avocado[13] and apples[14] and with substrate concentration in sweet potato[15], peaches[16], apple[17] and apricots[18]. It has been suggested that independent of the kind of fruit, the ratio of enzyme concentration and substrate might indicate which of these factors determined the browning rate of product[19]. Further, it is seen from studies reported that the endogenous level of ascorbic acid, a naturally occurring inhibitor of enzymatic browning, has been ignored as a factor that could influence the browning potential of fruit tissues. Ascorbic acid is known to act by reducing the quinones formed during enzymic oxidation back to phenolic compounds, the process being accompanied by a gradual decrease in PPO activity due to reaction inactivation[20].

In the present studies, 'Pachabale' and 'Poojabale' bananas which showed higher susceptibility to browning had relatively high PPO activity and concentration with low ascorbic acid content. 'Puttabale', which was least susceptible, had relatively low PPO activity and level and high ascorbic acid content. 'Rasabale', which had low susceptibility like 'Puttabale', had intermediate PPO activity and PPO level but relatively high ascorbic acid content, while 'Kadubale' with a slight browning tendency had low PPO activity and PPO level and low ascorbic acid content. It may, therefore, be concluded that varieties low in PPO activity and PPO level and high in ascorbic acid exhibited the least browning. As the five varieties did not show any significant differences in their total phenolic content, this did not appear to be a factor limiting browning in bananas.

From his studies using banana peel extracts, Griffiths[21] reported that dopamine was a characteristic product of the genome of *Musa acuminata* (*A*), but not of *Musa balbisiana*(*B*), the two main wild species from which majority of the edible bananas had their origin[5]. He therefore suggested that AA and AAA clones blackened strongly while hybrids AB, AAB, ABB and ABBB blackened less in proportion and *M. balbisiana* itself(BB) blackened very little. He did not, however, study the influence of PPO level.

In our study, while the cultivar 'Pachabale' (AAA) proved to be highly susceptible to browning, there was considerable dissimilairty in the browning potential among the hybrid varieties, 'Poojabale' (AAB) being nearly as susceptible as 'Pachabale' 'Kadubale' (AAB) being considerably less and 'Rasabale' (AAB) and 'Puttabale' (AB) being least susceptible suggesting no correlation with the genome or substrate concentration.

Our findings also differ from those of Weaver and Charley[22] who determined the dopamine and ascorbic acid content in the pulp of banana as it ripened and concluded that the concentration of dopamine, influenced in part by the concentration of ascorbic acid, might be a contributing factor in the browning of bananas and PPO activity was not a limiting factor. Thomas and Nair[23] on the other hand, found a good correlation between PPO activity and discolouration in irradiated banana fruit.

The natural pH of fruit tissues has not so far been implicated as a factor that could influence their browning potential. It is known that PPO from different plant sources exhibit different pH optima. The optimum in many cases is different from the natural pH of the pulp eg. grape PPO[24]. In the present studies on banana varieties, at the pH of 5.2 associated with the relatively highly susceptible 'Pachabale', the PPO exhibited about 57.5 per cent of its activity at the optimal pH of 7.0, while at pH 4.5 to 4.6 associated with the least susceptible 'Rasabale' and 'Puttabale' varieties and the slightly susceptible 'Kadubale', the enzyme showed only 6-11 per cent of the activity at the optimal pH. These results indicated that the natural pH of the fruit pulp was an additional factor influencing its browning potential, nearer the pH to the value optimum for the enzyme, with higher susceptibility due to increased activity of the enzyme.

Data on the variation of PPO activity and level with season in fruits and vegetables are meagre. Sato and Hasegawa[25] reported phenolase activity in spinach chloroplasts to gradually decrease from autumn to winter. The present observation with banana varieties showing a sharp increase in PPO activity and PPO level during winter months is significant since it shows that seasonal variation is another factor that could be utilised to advantage in selecting raw material with least PPO activity and concentration to minimise browning during processing.

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Influence of Turmeric and Curcumin on Growth, Blood Constituents and Serum Enzymes in Rats

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The whole spice turmeric or curcumin fed to rats at doses normally consumed or at much higher doses (1.25-125 times) than normal human intake did not cause any adverse effect on growth, FER, RBC, WBC, differential counts or on the levels of blood constitutents like haemoglobin, total serum protein, albumin, globulin and serum aminotransferases and alkaline phosphatase. At the highest level tried viz. 10%, the FER was much lower than normal because of low diet intake.

Turmeric (*Curcuma longa* L.) is one of the most common spices in India used mainly for its colouring properties and partly for flavour. Many medicinal applications are also described for this spice in the indigenous system of medicine[1]. This spice was studied at this institute to find out its suitability as a colouring agent for hydrogenated fat. In the course of experiments conducted for testing its safety, it was observed that the growth rates of rats fed alcoholic extract of turmeric at 0.04 per cent level were similar to that of control animals[2–4]. Further studies showed that alcoholic extract of turmeric had no effect on endogenous levels of cholesterol in liver and serum. However, hypocholesterolemic activity was observed in rats fed cholesterol[5]. Later studies showed that the hypocholesterolemic activity of turmeric extract was due to curcumin, the colouring principle of turmeric[6,7]. These observations are in conformity with the cholerteic and hydrocholagoguic activities of curcumin[8,9].

Turmeric extracts have been shown to possess antimicrobial[10] and antiprotozoal activity[11], while curcumin was shown to have bacteriostatic activity against *Staphylococcus*[12].

The anti-inflammatory effect of sodium curcuminate was observed in experimental inflammation induced by carrageenin and formalin in rats[13]. The anti-inflammatory and anti-arthritic activity of volatile oil of turmeric was also observed in Freund's adjuvant induced arthritis in the rat[14].

As a part of systematic work on the role of spices in nutrition and also to establish its safety as a food additive in response to a directive from the Government of India as required by the Codex alimentarius, studies were carried out to examine the effect of both turmeric and curcumin on growth, blood constitutents and serum enzymes in rats. Results of these studies are described in this communication.

Materials and Methods

Turmeric (*Curcuma longa*) fingers (dry) were purchased from the local market and powdered to 40-50 mesh. Curcumin isolated from turmeric was purchased from a local firm. Male albino rats of the Wistar strain (30-40 g) were used in the experiments. Rats were individually caged and had free access to water and diet. The composition of the control diet was as described earlier[15]. The experimental diets containing turmeric and curcumin at desired levels were prepared by incorporating them into the respective diets and mixed in a mechanical mixer. The rats were given weighed quantities of the diet and their daily food intake was calculated by subtracting the residual diet from the diet given. The rats were weighed weekly and the food efficiency ratio (FER) was determined for 4 and 8 weeks. Total serum protein was determined according to Gornall *et al*[16]. and albumin and globulin by Kingsley's procedure[17]. Blood glucose was determined by Nelson and Somogyi method[18]. Haemoglobin, RBC, WBC and differential counts were determined in the blood drawn by tail venipuncture[19]. Aminotransferases (glutamic oxaloacetic and glutamic pyruvic) and alkaline phosphatase were determined according to Tanhazy *et al.* and Hubschar *et al.* respectively[20,21].

Results and Discussion

The data on FER are given in Table 1. The food

TABLE 1. INFLUENCE OF TURMERIC AND CURCUMIN ON FOOD EFFICIENCY RATIO IN RATS[a]

% turmeric in the diet	Relative PER*		% Curcumin in the diet	Relative PER*	
	4th week	8th week		4th week	8th week
0	1.00 (0.342±0.009)	1.00 (0.279±0.005)	0	1.00 (0.306±0.008)	1.00 (0.239±0.006)
0.1	0.95	0.98	0.1	0.92	0.99
0.2	0.93	0.98	0.5	0.94	1.01
0.5	0.94	0.91	1.0	0.91	0.96
1.0	1.00	1.02	2.0	0.94	0.96
5.0	0.90	0.74			
10.0	0.49	0.55			

[a] All observations are the average of 10 values.

* FER on control diet has been taken to be 1.000, and the other values calculated on this basis; numbers in parenthesis are absolute FERs (g. gain in body weight/g. food intake).

intake, gain in weight, and FER of rats fed at different levels of turmeric and curcumin except at 10 per cent level of turmeric do not show any significant difference from controls. A significant decrease is found in food intake and weight gain in 10 per cent turmeric fed rats at 4 and 8 weeks. This lower growth and FER was mainly due to the lower food intake because of the unpalatability of the diet. Infact, rats fed 20 per cent turmeric in the diet had to be discarded as the food intake was very low because of the strong aroma and bitterness imparted, to the diet by the whole spice.

Feeding curcumin at any level shown in Table 2 did not make any difference to the haematological picture. Whereas no changes were observed in per cent haemoglobin and differential counts in animals fed turmeric, RBC was higher in groups fed 0.2 and 1.0 per cent turmeric and it was lower in the 10.0 per cent turmeric group. Though these changes were statistically significant, the values are within the normal range. As seen from Table 3, serum total protein, albumin/globulin ratio and blood sugar levels did not show marked deviations from normal except for a reduction in A/G

TABLE 2. BLOOD PICTURE OF RATS FED TURMERIC AND CURCUMIN

	% turmeric in the diet[a]				% curcumin in the diet[a]				
	0	0.2	1.0	10.0	0	0.1	0.5	1.0	2.0
Haemoglobin (g%)	16.4±0.26	16.8±0.26	16.3±0.26	15.5±0.26	16.9±0.19	17.1±0.23	15.0±0.28	17.1±0.45	16.9±0.33
RBC (mill./mm³)	7.4±0.19	8.3±0.19*	8.5±0.19*	6.06±0.19*	7.3±0.09	7.3±0.13	6.8±0.20	7.6±0.22	7.6±0.21
WBC (Counts per mm³×10³)	13.9±1.3	14.1±1.3	16.0±1.3	12.6±1.3	18.0±2.99	14.4±0.69	13.8±0.23	18.0±1.67	16.5±0.39
Differential counts (%)									
Polymorphs	26	22	22	19	20	17	23	21	24
Lymphocytes	66	67	68	71	75	75	72	74	70
Monocytes	6	9	7	8	2	3	2	2	3
Eosinophils	2	2	3	2	3	6	3	3	3

[a] Values are mean±SEM of ten animals in each group.

* $P<0.05$.

TABLE 3. EFFECT OF TURMERIC AND CURCUMIN ON BLOOD CONSTITUENTS

	% turmeric in the diet[a]			% curcumin in the diet[a]				
	0	0.2	1.0	0	0.1	0.5	1.0	2.0
Total proteins (g/100 ml)	6.2±0.42	6.7±0.42	6.5±0.42	5.9±0.27	6.5±0.28	5.8±0.16	5.9±1.30	6.2±0.32
Albumin/globulin ratio	1.16±0.48	1.02±0.48	2.34±0.48	1.97±0.23	1.13±0.12*	1.66±0.21	1.63±1.13	1.37±0.15
Glucose (mg/100 ml)	115±5.8	114±5.8	128±5.8	109±5.2	126±7.1	122±6.8	111±3.8	101±7.0

a Values are mean±SEM of 10 animls in each group.

* $P<0.05$.

ratio at 0.1 per cent curcumin. In all the groups of rats fed turmeric or curcumin, serum GOT, GPT and alkaline phosphatase activities did not show any significant deviation from the controls (Table 4). As it is known that serum aminotransferase levels are enhanced when there is inflammation, degeneration and neoplastic lesions of the liver as well as in heart diseases[22], it may be inferred from the above data that no such adverse effect was caused by feeding turmeric or curcumin.

At the time our experiments were planned, no information was available regarding the normal intake of turmeric. After analysing 6 different curry powders (a powder of a mixture of several spices) 4 g per adult per day of turmeric intake was arrived at. Later, a survey conducted by the National Nutritional Monitoring Bureau, Hyderabad (India) indicated a range of 0.1-3.8 g/adult/day. Further the FAO/WHO Expert Committee on Food Additives allocated an acceptable daily intake (ADI) of 0-2.5 mg/kg to turmeric and 0-0.1 mg/kg to curcumin[23]. The levels at which turmeric and curcumin were fed to rats in our study are 2-125 times higher than the 4 g/adult/day estimate, and 40-4000 and 1000-20000 times for turmeric and curcumin respectively of the FAO/WHO recommendations. No mortality was observed in any of the groups and no histopathological abnormality was noticed in gastro-interestinal tract, liver, spleen and kidney. At 10 per cent turmeric, the diet was unpalatable and the FER was therefore markedly lower. At all other levels no adverse influence was seen on FER, blood picture and serum aminotransferases. Similar conclusions have been arrived at in a parallel study in which acute toxicity studies were conducted in three dieffrent species of animals, on turmeric and its alcoholic extract[24].

TABLE 4. EFFECT OF TURMERIC AND CURCUMIN ON SERUM ENZYMES*

Addition to the diet(%)	GOT[a]	GPT[a]	Alkaline phosphatase[b]
0	117±9.34	28.6±3.01	7.08±0.67
Turmeric (0.1)	122±9.06	28.3±2.94	8.21±0.44
Turmeric (0.5)	105±5.8	27.2±2.26	9.38±1.23
Turmeric (5.0)	118±9.79	29.6±3.45	6.78±0.62
Curcumin (0.5)	119±7.33	26.0±2.98	6.86±0.55

* Values are mean±SEM of 10 animals in each group.

[a] μ moles of pyruvate formed/10 min./100 ml serum.

[b] μ moles of pi liberated/15 min./ml serum.

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Changes in the Composition of Clove Oil During Maturation

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Clove oil obtained at lower maturity stages of clove is found to have relativity more concentration of eugenol acetate and less of eugenol. With maturation of clove the eugenol acetate concentration decreases and the eugenol concentration increases. The changes affect the flavour and physicochemical characteristics of the oil. Generally, oil from fresh raw clove has a pleasant fruity odour.

Literature so far available on chemical composition of clove (*Eugenia caryophyllata* Thumb) refers to the fully mature and dried buds as sold in market. Early workers[1,2] reported mainly the general composition of the spice. Guenther[3] extensively described the volatile oil which is the predominant flavour principle of clove and listed eugenol, eugenol acetate and caryophyllene as the major constituents. This has been recently scrutinised by modern instrumental analysis using gas chromatography and mass spectroscopy[4,5]. Nearly 30 chemical components have so far been reported, some of them tentatively, in clove oil by the various workers. Some of the sugars[6], acids[7,8] steroid glycosides[9] and phenolics[10] have been studied in recent years.

However, no study has been made on the physicochemical characteristics of fresh cloves; the present

study deals with the quality characteristics and chemical profile of the essential oil at different maturity levels of clove.

Materials and Methods

Fresh cloves obtained from an estate in Trivandrum were used. The flowering season of clove in this region is generally October-November and the buds reach the mature stage in about four months. Cloves of 2, 3 and 4 months' (fully grown) maturity were collected and their individual physical characteristics were noted. The average weight/length of a bud was calculated from the values of 20 buds from each group. The buds of uniform maturity were crushed and homogeneously mixed for analysis. Moisture was determined by toluene distillation method and volatile oil by Clevenger distillation method[11]. Total hydrolysable carbohydrate was determined by acid hydrolysis followed by Lane-Eynon procedure[12]. Nonvolatile ether extract, crude fibre, crude protein and polyphenols (tannins) were determined as per standard AOAC[13] methods.

Volatile oil from the samples was collected by steam distillation and their physical characteristics determined as per ISI[14] methods.

The chemical profile of the volatile oil was determined by a Hewlet-Packward 5840 A gas chromatograph equipped with an intergrator using high performance carbowax column, 6 ft×1/8 in at 100-190°C @ 10°/min, with nitrogen as carrier gas at a flow rate of 20 ml/min, injector temperature at 300°C and FID detector at 300°C. The major peaks were identified by comparison of their retention times with those of authentic samples. The amounts of the major volatiles were calculated as the average of the values of duplicate analysis of integrated area percentages.

Results and Discussion

The physical and chemical composition of clove buds at different stages of maturity is given in Table 1. The concentration of volatile oil and nonvolatile ether extract (NVEE) is maximum at the lower maturity stages with slight decrease in the last phase. Dry weight of the bud continued to increase until the final growth. Synthesis of volatile oil is very high during the second and third months compared to later stages, while NVEE synthesis continued throughout the maturation period.

The concentration of polyphenols on dry weight basis remains steady throughout the maturation, even though polyphenolic constituents as identified by their adsorbing capacity to gelation (true tannins) showed a decrease. It is the latter, which is responsible for the astringent taste of a plant product.

TABLE 1. CHEMICAL COMPOSITION* OF CLOVE AT DIFFERENT MATURITY STAGES

Composition	2 months		3 months		3 months	
	(%)	per bud (mg)	(%)	per bud (mg)	(%)	per bud (mg)
Moisture (wet)	67.6	—	68.6	—	71.3	—
Av. bud wet. wet	—	157.8	—	250.1	—	305.8
dry	—	51.0	—	78.3	—	87.7
Av. bud length wet (cm)	—	1.25	—	1.58	—	1.74
Volatile oil	18.5	94.6	18.8	147.4	16.7	146.6
Nonvolatile ether extract	12.5	64.0	11.2	88.2	11.6	101.9
Total hydrolysable carbohydrate (as starch)	7.3	37.3	8.7	68.7	11.5	101.1
Crude fibre	12.4	63.5	12.5	98.1	15.9	139.8
Crude protein	6.8	35.1	6.9	53.8	6.9	60.4
Ash	5.4	27.5	5.6	43.9	6.5	57.7
Polyphenols**						
Total tannins	22.5	114.5	22.2	173.7	30.6	180.2
True tannins	13.6	69.2	11.3	88.7	8.5	78.4
By Polin Denis method	14.9	75.8	14.4	112.6	14.0	123.1

**By Lowenthal-Procter method (as quercitannic acid) * Dry weight basis

TABLE 2. PHYSICO-CHEMICAL CHARACTERISTICS OF CLOVE OIL AT DIFFERENT MATURITY STAGES

	Maturity stage					
	2 months	per bud (mg)	3 months	per bud (mg)	4 months	per bud (mg)
Specific gravity (27 C)	1.0244		1.0290		1.0395	
Refractive index (26 C)	1.5215	—	1.5225	—	1.5264	—
Specific rotation in MeOH (23 C)	-6.36	—	-8.76	—	-9.04	—
Eugenol	48.2%	45.5	54.3%	80.0	68.3%	101.1
Eugenol acetate	26.0%	24.5	21.0%	30.9	9.0%	13.1
Caryophyllene	10.9%	10.3	12.1%	17.8	11.2%	16.4

Among the other constituents, the total hydrolysable carbohydrate, crude fibre and ash increased towards the later stage while the concentration of protein showed a steady trend. However, in all cases, in view of the increase in dry weight of bud, there is significant increase in amounts per bud indicating continued steady synthesis.

Analysis of essential oil obtained at different stages of maturity (Table 2) revealed an increase in eugenol and decrease in eugenol acetate concentration. While the volatile oil from fully mature buds gave the characteristic harsh burning odour, contributed by phenolic essential oil constitutents, at a younger stage the oil had a more mellow odour. The caryophyllene concentration remained more or less steady. Essential oil distilled from fresh clove was found to have a pleasant fruity odour and the possible presence of ethylbutyrate and amylacetate in the oil has been indicated by GLC analysis.

The variation in composition of the oil invariably effected the physical characteristics. The refractive index and specific gravity exhibited a gradual increase, while specific rotation showed a slightly more significant increase with maturity.

Even on per bud basis, eugenol acetate showed a decrease especially in later stages indicating disappearance of the constituent and possible conversion to eugenol. Caryophyllene showed an increase in concentration per bud in the early growth phase and subsequently remained steady

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Formulation of a Weaning Food with Low Hot-paste Viscosity based on Malted Ragi *(Eleusine coracana)* and Green gram *(Phaseolus radiatus)*

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Ragi (*Eleusine coracana*) and green gram (*Phaseolus radiatus*) were steeped in water for 16 hr, and germinated for 48 and 24 hr respectively, dried and powdered after removal of the vegetative portion and bran. Refined ragi flour was mixed with green gram flour in the ratio of 70:30 to produce a malted weaning food (MWF). Significant increase in amylase activity and decrease in paste viscosity occurred with progressive germination of ragi and green gram. Ragi showed higher enzymic and viscosity changes than green gram. Hot paste viscosity of MWF was much lower than that of several proprietary brands of weaning foods manufactured in India. Reduction of the paste viscosity of weaning foods marketed in India can be effected by the use of barley malt flour.

The usefulness of weaning foods to meet the nutrient needs of children being weaned from a liquid diet (milk) to a soft semi-solid or solid diet is now well recognised, and several types of weaning foods have been formulated in India and in different parts of the world[1]. The popular weaning foods marketed in India are very costly and beyond the reach of the majority of the population. Also, most of these are precooked roller dried mixtures of cereal and/or legume flours and possess a high viscosity or high bulk when stirred with hot or cold water which limits the total food intake by the child. Hence, emphasis is now being placed by nutritionists on weaning foods with low paste viscosity or high calorie density[2].

The effect of traditional cereal processing techniques used in India on the paste viscosity of gruels indicated that malting/germination produced a maximum fall in the viscosity as compared with many other heat treatments[3]. Apart from this, malting has many nutritional benefits such as increase in vitamin[4,5], lysine and tryptophan contents[6-9]. The malting process was therefore, made use of in formulation of a low cost weaning food of low viscosity and high calorie density. Also, a simple technique based on the use of malt flour was developed to reduce the paste viscosity of Indian proprietary weaning foods. The results are reported in this paper.

Materials and Methods

Selection of millet and legume as ingredients for malt food: Maize (*Zea mays*), Jowar (*Sorghum vulgare*), Bajra (*Pennisetum typhoideum*), Ragi (*Eleusine coracana*) and Navane (*Setaria italica*) seeds were germinated as described earlier[10]. Samples were removed each day, dried in hot air at 65°C and amylase activity in the malted whole meal flours was determined as per method of Bernfeld[11]. As ragi presented some advantage such as good malted flavour, high calcium content and relatively lower price, it was chosen for further detailed studies. Green gram was selected as the legume component for mixing with the ragi in view of its low flatus[12] and anti-trypsin factor[13].

Standardisation of conditions for germination of ragi and green gram and preparation of malt: The grains were soaked in distilled water at 25°C for different periods upto 40 hr. Samples were withdrawn at suitable intervals and moisture content and per cent of germinated grains were determined. Moisture was determined by oven drying method while germination was carried out in a B.O.D. incubator at 25°C. For malting studies, germination of ragi and green gram was done for 48 and 24 hr respectively, after which the materials were dried in a hot air oven at 65°C, and shoots and rootlets were removed by hand rubbing. The bran from green gram was removed by abrasive milling in a 'Rice Huller' mill, while bran was removed from ragi after moist conditioning and grinding in a plate grinder as described earlier[14]. A malted weaning food (MWF) was formulated by blending malted ragi and green gram flours in the proportion of 70:30.

Effect of malting on viscosity of gruel: Malted ragi and green gram flours were reconstituted in water at

different solid concentrations (2.5 to 25 per cent), heated for 20 min on a boiling waterbath, cooled to ambient temperature and the viscosity was measured in a Brookfield Viscometer (LVT model), with r.p.m. of 60 and varying appropriate spindles. The comparative viscosity of MWF as well as 4 brands of proprietary weaning foods was determined similarly. The effect of addition of malted barley powder (at 5 per cent level) to the proprietary weaning foods on their paste viscosity after adding boiling water to dry mix and stirring for 10 min was also determined.

Amyloviscography of 15 per cent slurries of different weaning foods from market and their blends with barley malt flour was also carried out in a Brabender amyloviscograph (VSK-4 model) using the method of Hallic and Kelly[15]. The proximate composition of the MWF was determined by standard analytical methods[16], while that of proprietary weaning foods are collected from the composition displayed on unit pack tins.

Results and Discussion

Amylase activity of millets increase with period of germination (Table 1). It was higher in bajra and ragi at 48 hr of germination, although at 96 hr of germination sorghum gave high activity. Longer period of germination about 48 hr caused the emergence of longer rootlets which resulted in greater loss of solid matter. Although high in amylase activity, the malt from bajra was found to have an undesirable smell and developed highly bitter taste after few days of storage because of its high lipase content. Similar observation was also made by Pal *et al*[17]. For these reasons, ragi was chosen for further detailed studies in the present work. Other grains may be used only where ragi is not available.

Viscosity characteristics of malted ragi, green gram and weaning foods: As can be seen from Fig. 1 the optimum soaking time for maximum germination was 16 hr for ragi and 10 hr for green gram, at which time the moisture content in the grains was 35 and 55 per cent respectively, and this soaking period was used in further studies. The effect of different periods of germi-

TABLE 1. ELABORATION OF AMYLASE ACTIVITY* DURING GERMINATION OF MILLETS

Sample	Period of germination (hr)			
	24	48	72	96
Ragi	25	64	178	150
Bajra	52	110	125	115
Sorghum	15	50	158	185
Maize	10	45	80	120
Navane	12	38	114	105

*Mg of maltose released by 1 g of malt at 37°C when acted on 1 ml of 1 per cent starch substrate for 30 min.

TABLE 2. PROXIMATE COMPOSITION OF WEANING FOODS (PER 100 G)

	Malted weaning food*	Commercial brands of weaning fords			
		1	2	3	4
Moisture, (%)	6.0	3.5	5.0	2.2	4.0
Protein, (g) (N×6.25)	11.5	12.0	7.5	11.0	22.0
Fat, (g)	1.5	3.0	—	7.8	3.5
Carbohydrates, (g) (by diff.)	76.7	75.0	84.0	77.0	68.0
Crude fibre, (g)	2.0	—	—	—	—
Total ash, (g)	2.3	3.5	3.0	2.0	—
Calcium, (mg)	240.0	750.0	690.0	275.0	800.0
Phosphorus, (mg)	210.0	400.0	570.0	225.0	690.0
Calorie	396	350	366	422	380

* Plain mix without fortification

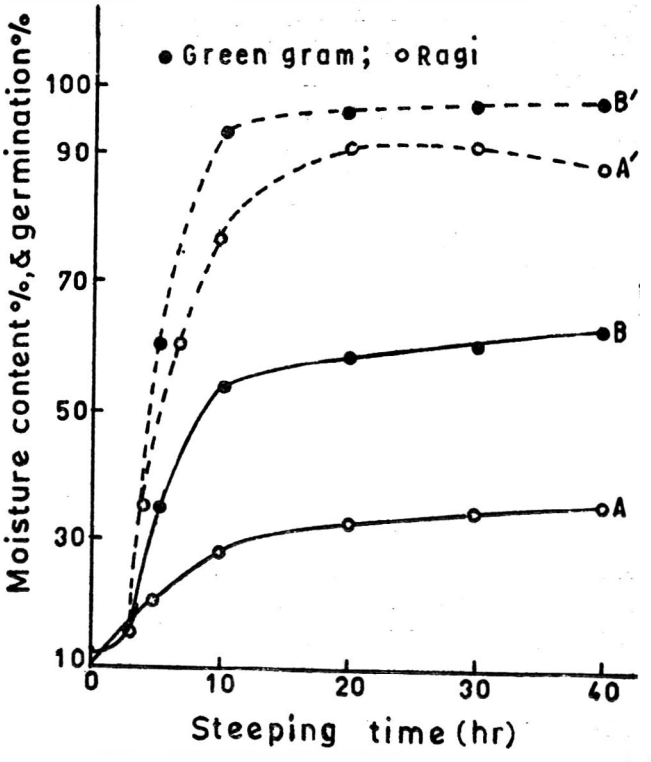

Fig. 1. Effect of steeping time on moisture content and germination percentage in ragi and green gram.
A & B —Moisture content
A' & B'—Germination (%)

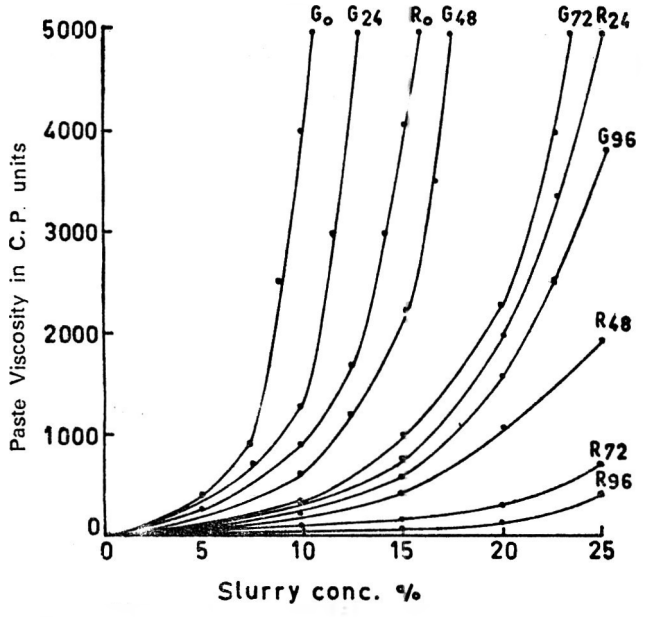

Fig. 2. Effect of period of germination on paste viscosity of ragi and green gram malt.

G—Green gram,
R—Ragi. Subscripts refer to hours of germination.

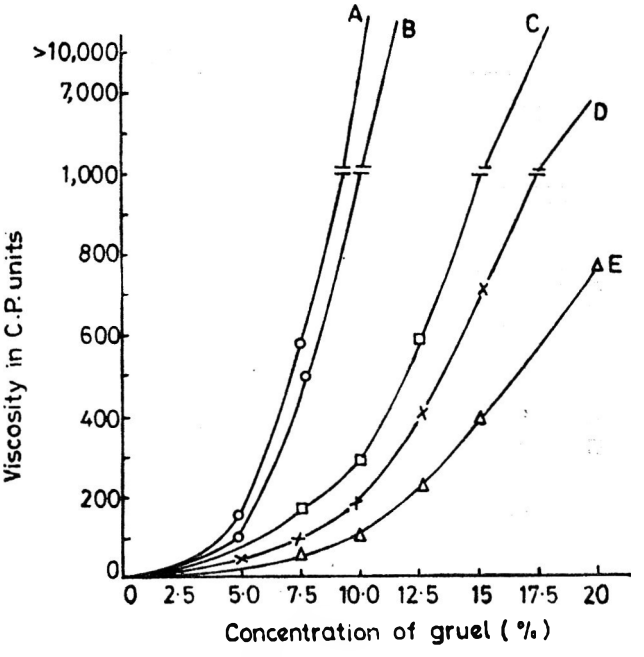

Fig. 3. Paste viscosity of weaning foods at different slurry concentrations.

A—Nestum; B—Farex; C—Cerelac;
D—Balamul; E—MWF.

nation of ragi and green gram on viscosity of gruels prepared from their respective malt flours is presented in Fig. 2. It is clear that reduction in paste viscosity increases with progressive germination. The fall in viscosity at all comparable slurry concentrations was greater in ragi than in green gram. For instance, after 48 hr germination and at 15 per cent slurry concentration, there was reduction in viscosity in case of ragi by about 1/10 while for green gram the reduction was only 1/3. Prolonging the period of germination beyond 48 hr brought down the viscosity of ragi slurry to a very low level. Even at 20 per cent concentration level the slurry was thin and free flowing.

Comparison of the slurry viscosity of the MWF with the proprietary weaning foods (Fig. 3) shows that the MWF, at all concentrations, had considerably less viscosity than proprietary weaning foods indicating the highly beneficial effect of malting in reducing the paste viscosity and increasing the calorie density of foods. The viscosity of the MWF was even lower than that of Balamul, which is known to have a low viscosity because of its relatively lower content of cereals. For a comparable gruel viscosity of 500 Cp units, the MWF has 50-100 per cent more calories as compared with different proprietary weaning foods.

The difference in the viscographic characteristics of the different weaning foods at 15 per cent slurry concentrations is more clearly brought out in the amyloviscographic studies presented in Fig. 4. The proprietary

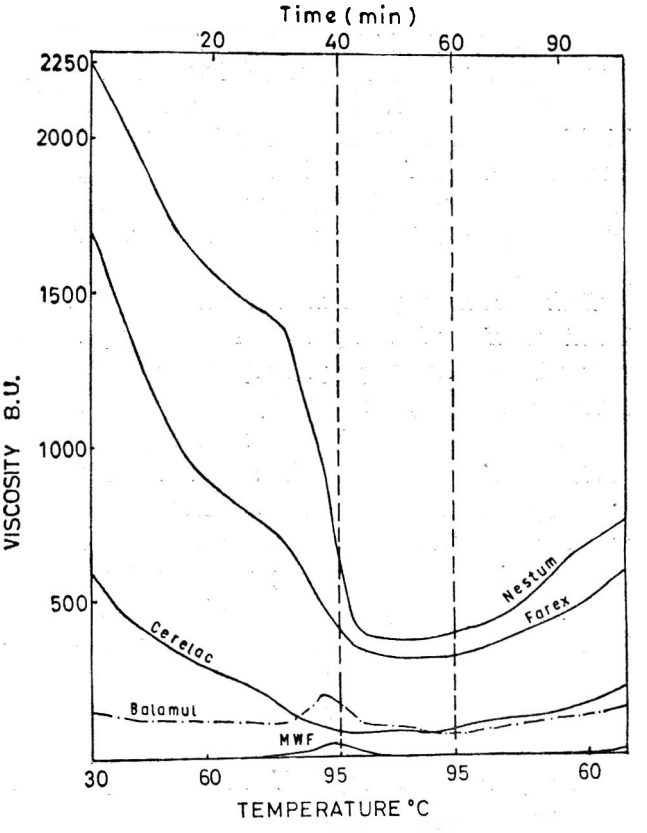

Fig. 4. Amyloviscograms of weaning foods at 15% slurry concentrations.

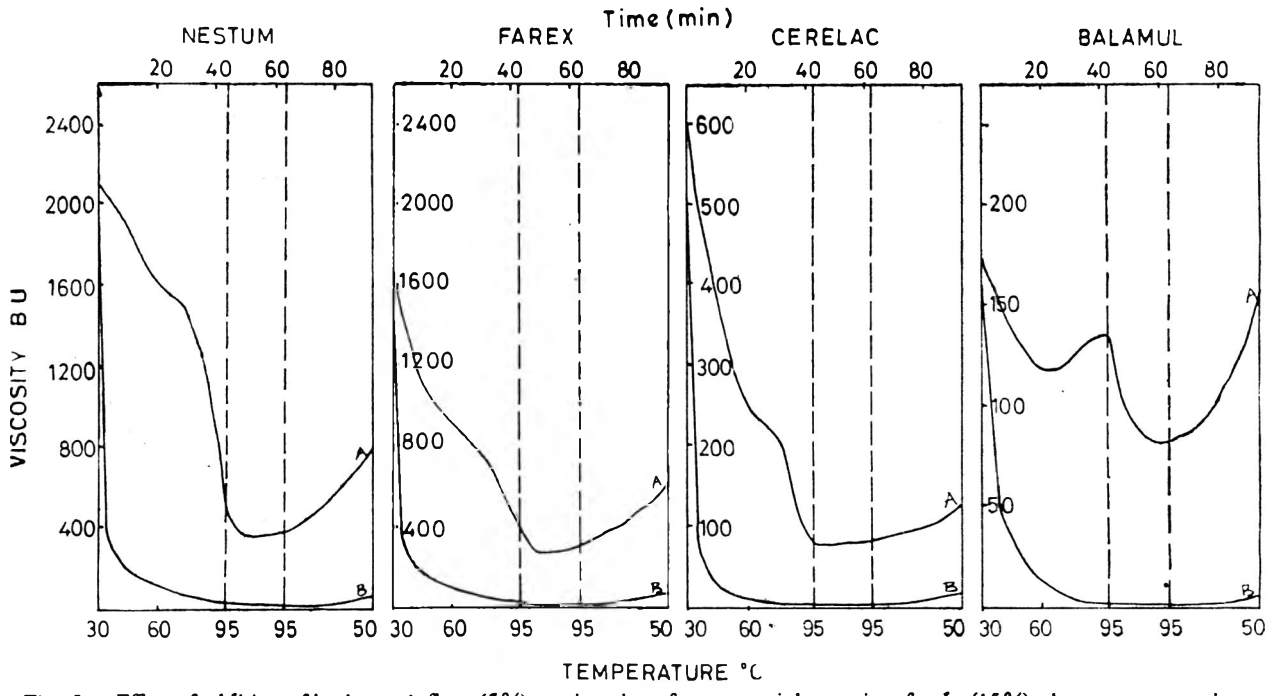

Fig. 5. Effect of addition of barley malt flour (5%) on viscosity of commercial weaning foods (15%) slurry concentration. A: Control; B: Improved.

weaning foods, being precooked starchy materials, have a high cold paste viscosity as compared with MWF which had negligible viscosity. At 95°C, the MWF had an extremely low viscosity and was almost a free flowing fluid, while the commercial weaning foods had very high viscosities. Even after cooling to 50°C the MWF remained thin while the proprietary weaning foods set to a thick gel. This clearly brings out the superiority of the MWF over roller dried weaning foods in terms of increased calorie density and reduced 'bulk' of the product[18].

'Bulk' reduction of commercial weaning foods: The beneficial effect of adding, even as little as 5 per cent of malted barley flour to proprietary weaning foods on the reduction of their paste viscosity is illustrated in Fig. 5. It is seen that considerable reduction in paste viscosity could be effected even within 5 min after heating or warming up. Such addition is therefore, feasible in weaning food formulations for increasing their calorie density. The reduced viscosity would enable the child to take more of the product per feed and total nutrient intake would be high. Earlier studies with amylase addition prior to drum drying of the ingredients during the preparation of the weaning foods have indicated problems of reduction in biological value of protein and reduced lysine availability[19]. This is not likely to be the case when barley malt flour is added as a source of amylase to the weaning food after it has been drum dried. Moreover, addition of barley malt flour to the weaning foods would not dilute the nutrients of these foods as malt flour has good quality protein[20].

Nutritional value: The proximate composition of MWF is similar to that of popular proprietary weaning foods (Table 2). It has 11.5 per cent protein and PER of 2.4[21,22]. Clinical trials indicated that the MWF has good tolerance and growth promoting values when fed to children. Results of these aspects will be published separately. Taking the prices of ragi and green gram as Rs. 2.00 and Rs. 5.00 per kg respectively, the approximate cost of production of MWF would be Rs. 6.00 per kg.

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Studies on Packaging and Storage of Fried Mung *(Phaseolus aureus)* Dhal

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Use of suitable flexible pouches and rigid containers (tin and aluminium) for packaging fried mung dhal (green gram dhal, *Phaseolus aureus*) greatly enhanced the shelf-life of the product. Fried mung dhal packed in different flexible packs and stored at 38°C and 92% RH indicated a shelf-life of 40, 60, 40 and 120 days with HDPE, MXXT+ LDPE, polyester/polyethylene vacuum pack and paper / polyethylene / aluminium foil/polyethylene respectively. The corresponding shelf life achieved with the above packings at 27°C and 65% RH were 110, 110, 110 and 210 days respectively. Fried mung dhal packed in tin and aluminium cans indicated a shelf life of more than a year under both storage conditions. Peroxide value was found to have no correlation with deterioration in flavour or storability.

Deep fat fried mung dhal (green gram, *Phaseolus aureus*) is a popular Indian savoury product. At present, fried dhal is being prepared on small scale by the sweetmeat manufacturers. Soaking the dhal overnight, draining and deep fat frying are the steps involved in the preparation. Fried mung dhal is known for its crisp

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texture and typical fried taste. It contains about 20 per cent oil, 18 per cent protein and 2 per cent moisture. About 1 per cent common salt is added to enhance the taste. The product is usually stored in glass jars or in high density polyethylene pouches. Under these conditions, the product has a shelf-life of about 15 days. Of late, a few entrepreneurs have shown interest to export the product to foreign market. This naturally needs extension of storage life.

Deteriorative changes, mainly due to exposure to atmosphere. are loss of flavour, development of rancidity and soft texture. These undesirable changes could be delayed by the use of appropriate packaging material. There is no information regarding the packaging of fried dhal. Polythene pouches are being used by some manufacturers. The present work was carried out with a view to find out the performance of flexible and rigid packaging materials for packaging fried mung dhal for domestic as well as export market.

Materials and Methods

Green gram dhal was soaked overnight, drained on a wire mesh sieve and fried in oil at 170°C. Refined palm oil was used for frying. Frying ratio of oil to material was about 10:1 and the frying time was about 1 min. Fried mung dhal, after draining was mixed with 1 per cent table salt containing butylated hydroxy anisole (BHA). Concentration of BHA in the salt was adjusted in such a way that the finished product contained 0.02 per cent BHA on its fat content.

Sorption-isotherm studies: To determine the equilibrium relative humidity (ERH) of fried mung dhal, the sorption isotherm studies were carried out at 27°C. Samples in lots of 5-6 g in duplicate were exposed to humidities ranging from 11 to 92 per cent build up in desiccators by using appropriate saturated salt solutions. The samples were weighed periodically till they attained constant weight or the onset of mould growth whichever was earlier.

Packaging: Deterioration of fried dhal occurs due to loss of texture (absorption of moisture) and development of rancidity (absorption of oxygen). Accordingly, packaging materials were selected taking into account the above requirements besides the economic considerations and availability of the materials. Four flexible packaging materials namely (*i*) high density polyethylene (HDPE), (*ii*) double pouch of MXXT (inside) and low density polyethylene (LDPE) (outside), (*iii*) polyester/polyethylene vacuum pack and (*iv*) laminate consisting of paper/polyethylene/aluminium foil/polyethylene were selected. The water vapour transmission rates (WVTR) of these packaging materials were determined according to ISI method[1] and are expressed as g/m² at 90 per cent RH gradient and at 38°C. The results are given below.

	Material	WVTR, g/m² at 38°C and 90%RH
1.	300 G—HDPE	1.4
2.	MXXT cellophane	8.2
3.	200 G—LDPE	5.7
4.	50 G polyester/300 G LDPE laminate	4.9
5.	60 GSM paper/150 G—LDPE/ 0.009 mm Al. foil/150 G LDPE	*Nil*

Fried dhal was packed in unit packs containing 50 g of the sample.

For export purposes tin and aluminium containers were also selected. Tagger top tin cans of 8 oz capacity (220 ml) and a two-stage impact extruded shallow aluminium dish type containers of 3 oz capacity (90 ml) were used. The tagger top tin can had the regular flip open tin lid and the aluminium can had an appropriate size tin plate lid with a rubber lining compound. To the sealed tin containers containing dhal, nitrogen and carbon dioxide gases were injected under 26 in. vacuum in a gas filling unit and the hole in each can was sealed by spot soldering. Aluminium cans were closed with a double seamer and one set of cans were provided with inner polyethylene liner. Dhal samples were also packed in plain (just air and no N_2/CO_2) rigid containers.

Storage condition: The flexible packages were stored at 27°C and 65 per cent relative humidity (R.H.) which was the normal Indian climate and at 38°C and 92 per cent R.H. (accelerated condition). The product packed in glass bottles and flushed with nitrogen and stored at 5°C was used as reference material.

The rigid container samples were stored at 5°C, 27°C and 38°C. The samples in plain tin and aluminium containers and stored at 5°C served as reference material for tin and aluminium rigid containers respectively.

The samples in flexible packages stored at 38°C and 92 per cent R.H. were withdrawn after 20. 40, 60. 80, 100 and 120 days and those stored at 27°C and 65 per cent R.H. were withdrawn after 30, 50, 90, 110, 130, 150 and 210 days. The moisture pick up during storage was determined by weighing the pocuhes at each withdrawal.

The samples in rigid containers were withdrawn after 90, 180, 270 and 360 days of storage.

Analytical procedures: Samples were extracted with chloroform at ambient temperature. Aliquots from this extract were taken for estimation of peroxide value and free fatty acid content following the methods of A.O.C.S.[2]

Sensory evaluation: The quality attributes like colour, appearance, texture and flavour of fried dhal were chosen to study the samples kept in (*a*) 4 flexible packaging materials under 2 storage conditions; (*b*) one tin container under 9 storage conditions; and (*c*) one aluminium container under 4 storage conditions. Ranking

TABLE 1. QUALITY DESCRIPTION OF FRIED GREEN GRAM DHAL

Quality attributes	Desirable	Undersirable
Colour and appearance	Bright, typical of fresh product; slight bloom	Dull: high bloom
Texture	Crisp & crumbly	Soft; soggy
Flavour (Aroma, taste and mouthfeel)	Characteristic fresh, fried flavour; slight oily feeling	Stale; rancid; high oily feeling

method was adopted and acceptability study of each treatment/sample in all evaluations was also carried out.

Twenty five staff members, familiar with fried dhal, were chosen as panelists. In two orientation sessions, the panelists were shown different dhal samples, quality of which varied from excellent to poor. The desirable and undesirable qualities for each attribute were arrived at after discussion for regular evaluation (Table 1).

The evaluations were carried out as per the I.S.I. recommended laboratory set up[3] in air-conditioned room (22°C). Five samples (4 types of packages under one storage condition and one reference sample) were evaluated in each session when flexible package samples were evaluated. When rigid container samples were evaluated, either samples in tin containers (at 3 different temperatures under one gas/at 3 different gases at one temperature and one reference sample) or 5 samples in aluminium containers (at 2 different temperatures with/without polyliner lining and one reference sample) were evaluated in each session. The evaluation results were analysed by rank sum analysis and 2-tailed binominal probability test[4].

Results and Discussion

Table 2 gives the chemical composition of four commercial samples of fried mung dhal. Moisture content varied from 1.3 to 2.3 per cent; fat content from 20.7 to 24.0 per cent. Difference in fat content was mainly due to variations in frying conditions and varietal difference of mung dhal. Very little fat (about 0.5 per cent) was in the bound form. Protein content was from 17.4 to 19.8 per cent and starch content varied from 45.5 to 46.7 per cent.

TABLE 2. COMPOSITION OF FRIED MUNG DHAL (COMMERCIAL SAMPLES)

Sample No.	Moisture (%)	Fat (%)	Bound fat (%)	Protein (%)	Starch (%)
1	2.3	24.0	0.7	17.4	45.6
2	2.2	20.7	0.4	19.4	46.7
3	1.3	24.0	0.5	18.8	45.5
4*	1.8	21.8	0.6	19.8	45.9

*Sample used in the present study

Fried dhal had a moisture content of 1.8 per cent which corresponds to 7 per cent R.H. (Fig. 1). The sorption isotherm showed the tendency of the product to absorb moisture. The product lost its crisp texture when moisture content was 4-6 per cent or above (the critical moisture content.) Therefore, any packaging material used should have low water vapour transmission rate.

Moisture barrier property of the different flexible packages is shown in Table 3. At 27°C, the initial rate of increase of moisture content in HDPE and MXXT+LDPE pouches was low compared to polyester/PE pouch; however, by 210 days of storage, dhal packed in all the three types of packages showed equal moisture content. At 38°C storage, HDPE showed lowest increase in moisture content. Samples in aluminium foil laminate did not show any moisture pick up at both the storage temperatures even upto the last day of storage.

Tables 4 and 5 give the peroxide value (PV) during storage of flexible and rigid container samples respecti-

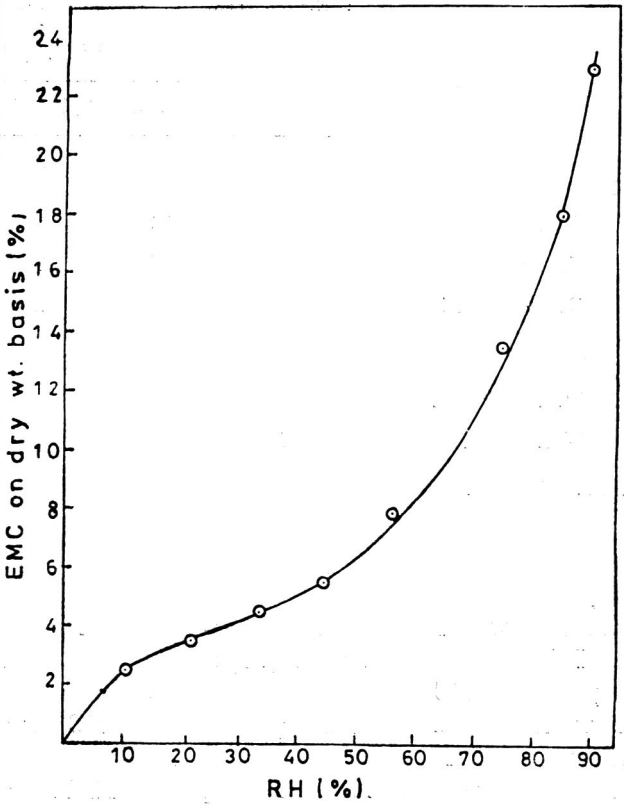

Fig. 1. Sorption isotherm for fried mung dhal at 27°C

TABLE 3. MOISTURE CONTENT OF FRIED MUNG DHAL DURING STORAGE (INITIAL MOISTURE CONTENT 1.8%)

Packaging material	Storage period (days) at 27°C, 65% RH							Storage period (days) at 38°C, 92% RH					
	30	50	90	110	130	150	210	20	40	60	80	100	120
HDPE	2.1	2.4	2.8	3.1	3.3	3.6	4.6	2.8	3.9	4.7	5.5	6.2	7.1
MXXT+LDPE	2.2	2.4	2.8	3.2	3.4	3.8	4.6	3.2	4.6	5.6	6.5	7.4	8.5
Polyester/PE	2.4	2.8	3.3	3.7	4.0	4.4	4.6	3.6	5.2	6.4	7.4	8.2	9.0
Paper/PE/Foil/PE	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8

TABLE 4. PEROXIDE VALUE OF FRIED MUNG DHAL STORED IN FLEXIBLE PACKAGES

Packaging material	PV (meq O_2/kg oil) during indicated storage period (days)												
	27°C; 65% R.H.							38°C; 92% R. H.					
	30	50	90	110	130	150	210	20	40	60	80	110	120
HDPE	1.6	2.8	3.5	3.5	3.9	3.8	3.0	1.7	5.4	7.2	6.1	3.6	5.6
MXXT + LDPE	2.1	2.5	2.7	2.7	3.2	3.0	3.0	2.0	5.3	7.0	7.2	3.1	5.0
Polyester/PE	1.2	1.8	2.8	2.8	2.9	2.6	3.5	1.5	4.3	4.5	5.0	1.9	1.9
Paper/PE/foil/PE	1.9	1.9	2.0	2.1	2.4	2.1	2.5	1.7	4.4	4.0	4.1	2.6	2.8

Initial PV was 0.

TABLE 5. PEROXIDE VALUE OF FRIED MUNG DHAL STORED IN RIGID PACKAGES

Packaging material	PV (meq O_2/kg oil) during indicated storage period (days)							
	27°C, 65% R.H.				38°C 92% R.H.			
	90	180	270	360	90	180	270	360
Tin								
Plain	2.0	2.5	2.5	2.5	2.0	4.0	4.5	4.0
Nitrogen	1.5	1.5	1.8	1.5	1.5	2.5	2.0	2.0
Carbon dioxide	1.5	1.5	2.0	2.0	1.5	2.5	2.5	2.8
Aluminium								
Without PE liner	1.5	1.5	2.5	2.5	2.0	3.5	4.0	4.0
With PE liner	1.5	1.5	2.5	2.5	2.0	3.5	4.0	4.0

Initial PV was 0.

vely. Samples in rigid containers and in foil laminate had the lowest PV at both the temperatures. At 27°C, the PV reached the maximum (3.9) between 110 and 130 days and at 38°C, the maximum value (7.2) was reached between 60 and 80 days. Samples stored at 38°C always showed higher PV than those stored at 27°C throughout the storage period. At 38°C, samples in HDPE and MXXT+LDPE showed higher PV than those packed in polyester/PE and Al. foil based laminate. This may be ascribed to lower oxygen availability in samples packed in polyester/PE and Al. foil based laminate. At 38°C, the peroxide values in cans filled with CO_2 and nitrogen were lower (2.8 and 2.5) than those in aluminium foil based laminate as well as in control can. Inert atmosphere inside the can may be responsible for the lower values for peroxides.

TABLE 6. FREE FATTY ACID CONTENT OF FRIED MUNG DHAL STORED IN FLEXIBLE PACKAGES

Packaging material	FFA (as % oleic acid) during indicated storage period (days)											
	27°C, 65% R.H.						38°C, 92% R.H.					
	30	50	90	130	150	210	20	40	60	80	100	120
HDPE	0.12	0.12	0.15	0.19	0.20	0.27	0.11	0.13	0.13	0.15	0.19	0.24
MXXT + LDPE	0.11	0.12	0.17	0.19	0.21	0.25	0.10	0.14	0.14	0.15	0.18	0.24
Polyester/PE	0.11	0.12	0.14	0.16	0.18	0.25	0.11	0.15	0.14	0.14	0.16	0.19
Paper/PE/foil/PE	0.11	0.11	0.13	0.15	0.15	0.21	0.10	0.13	0.13	0.14	0.14	0.16

Initial FFA was 0.10% oleic acid.

TABLE 7. FREE FATTY ACID CONTENT OF FRIED MUNG DHAL STORED IN RIGID PACKAGES

Packaging material	FFA (as % oleic acid) during indicated storage period (days)							
	27°C, 65% R.H.				38°C, 92% R.H.			
	90	180	270	360	90	180	270	360
Tin								
Plain	0.13	0.13	0.15	0.15	0.13	0.15	0.15	0.15
Nitrogen	0.13	0.13	0.13	0.15	0.13	0.13	0.13	0.13
Carbon dioxide	0.13	0.14	0.14	0.15	0.13	0.13	0.15	0.15
Aluminium								
Without PE liner	0.13	0.14	0.15	0.15	0.13	0.15	0.15	0.15
With PE liner	0.13	0.14	0.15	0.15	0.13	0.15	0.15	0.15

Initial FFA was 0.10% oleic acid

TABLE 8. EFFECT OF PACKAGING—RANK SUM ANALYSIS AND ACCEPTABILITY OF FRIED MUNG DHAL DURING STORAGE AT 27°C AND 65% R.H.

Storage period (days)	Texture		Flavour		Acceptable
	Inf. to Ref.	Equal to Ref.	Inf. to Ref.	Equal to Ref.	
30	No. sig. diff.	No. sig. diff.	No. sig. diff.	Nol sig. diff.	ABCD
50	,,	,,	,,	,,	,,
90	,,	,,	,,	,,	,,
110	,,	,,	A	BCD	,,
130	BC	AD	ABC	D	D
150	ABC	D	ABC	D	D
210	ABC	D	ABC	D	D

Colour and appearance did not show significant difference during the entire storage period.

A: HDPE; B: MXXT+LDPE; C: Polyester/PE; D: Paper/PE/Foil/PE

Inf. to Ref. - Inferior to reference
Equal to Ref. - Equal to reference } $0.01 < P \leq 0.05$

TABLE 9. EFFECT OF PACKAGING—RANK SUM ANALYSIS AND ACCEPTABILITY OF FRIED MUNG DHAL DURING STORAGE AT 38°C AND 92% R.H.

Storage period (days)	Texture		Flavour		Acceptable
	Inf. to Ref.	Equal to Ref.	Inf. to Ref.	Equal to Ref.	
20	No. sig. diff.	No. sig. diff.	No. sig. diff.	No. sig. diff.	ABCD
40	,,	,,	,,	,,	,,
60	AC	BD	ABC	D	BD
80	ABC	D	ABC	D	D
100	ABC	D	ABC	D	D
120	ABC	D	ABC	D	D

Colour and appearance did not show significant difference during the entire storage period.

A: HDPE; B: MXXT+LDPE; C: Polyester/PE; D: Paper/PE/Foil/PE

Inf. to Ref.=Inferior to reference
Equal to Ref.=Equal to reference
} $0.01 < P \leq 0.05$

Tables 6 and 7 gives the changes in free fatty acid content of fried mung dhal in flexible and rigid packages respectively. There was no significant increase in free fatty acid content of samples at both the storage conditions in flexible and rigid containers.

The sensory evaluation data of flexible package samples are summarised in Tables 8 and 9. The reference sample maintained significantly superior quality in all the attributes and high acceptability throughout the storage studies. There was no significant difference in colour and appearance between all the treatments in both the storage conditions. At 38°C, in HDPE and polyester/PE packages, the samples could be stored upto 40 days while in MXXT+LDPE package the storage period was 60 days. Storage beyond these days resulted in an unacceptable product with soft texture and stale flavour. At 27°C, samples packed in HDPE, MXXT+LDPE and polyester/PE had storage life of 110 days. Samples packed in aluminium foil laminate were acceptable even after 210 and 120 days at 27 and 38°C respectively.

Dhal samples stored in tin containers showed no significant difference upto 360 days in colour and appearance, texture and flavour. It also indicated that temperature (5°, 27° and 38°C) and gas (plain, N_2 and CO_2) had no significant effect on product quality. Similar results were obtained in aluminium container samples also; temperature (27°C and 38°C) and inner polyethylene liner (with/without) showed no significant effect on dhal quality. Dhal stored in rigid containers even without nitrogen or carbon dioxide were acceptable at the end of 360 days. The conditions of all the rigid containers were normal and free from feathering, detinned spots or rusting.

In oils and fats, peroxide value of 10 was fixed as the upper limit[5]. In the present study, the products at 38°C showed a shelf life of 40 days in HDPE and polyester/PE, 60 days in MXXT+LDPE and more than 120 days in aluminium foil packs, but their corresponding PV were 5.4, 4.3, 7.0 and 2.8 respectively. Therefore, no correlation could be observed between PV and shelf life of the product.

Similar is the case with the products stored at 27°C also. They were acceptable upto 110 days in HDPE, MXXT+LDPE, polyester/PE and more than 210 days in Al. foil based laminate packs and the corresponding PV were 3.5, 2.7, 2.8 and 2.5 respectively. Though the PV did not exceed 4, yet the products became unacceptable in the case of HDPE, MXXT+LDPE and polyester/PE. No correlation could be observed between shelf life and PV. It may be noted in this connection that none of the products during entire period of storage either at 27°C or at 38°C reached a PV of 10 meq which is the prescribed limit for many of the edible oils. Hence, as an objective indicator of the acceptability of the product. PV may not be the best index for fried *mung dhal.*

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Effect of Castration/Sterilization on Eating Quality and Nutritive Value of Meat in Male Goats

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The eating quality and proximate composition of meat as affected by vasectomization and castration were studied in Alpine X Beetle crossbred goats. The scores obtained for tenderness, flavour and juiciness for intact goats were not different from those obtained for goats castrated (by burdizzo or removal of testes) or sterilized (by vasectomy) at the age of 2, 4 or 6 months. Moisture content of the meat was similar in intact, castrated and vasectomized goats. While protein level was slightly higher in the meat of intact goats, fat content was higher in castrated and sterilized goats. Digestibility with pepsin and trypsin of meat protein was higher in goats castrated by burdizzo.

The castration of animals involving the removal of sex glands results in retradation of growth. As a consequence of reducing the oxidizing process and increasing the assimilation in castrates, more fat is deposited. Changes also affect the nervous system; in the castrate there is some predominance of inhibiting processes over processes of exicitment, the violent temperaments and aggressiveness disappear making herd management easy. Experimental evidence on cattle, sheep and pigs show that castration of males generally improves the eating quality of resulting meat. Beef from steers is more tender than that from bulls[1-4]; meat from wether lamb has higher desirable flavour and tenderness than that from rams[5,6]; pork from intact boars is inferior to that from castrated boars and has an undersirable odour upon cooking[2,7].

Goat is a principal meat animal in countries like India, and no information is available on the effect of castration or sterilization of male goat kids on the quality of its meat. The present work reports the effect of partial and complete castration on eating and nutritional quality of meat in male goats.

Materials and Methods

The studies were carried out on Alpine X Beetle crossbred male kids of goats (obtained from National Dairy Research Institute, Karnal). Animals of three different age groups (2,4 and 6 months) were vasectomized or castrated by burdizzo and surgical removal of the tests. In vasectomy, after exposing through opening the anterior portion of the scrotum, the vas deferens was ligated in two places leaving a space of 1-2 cm., cut and removed. In burdizzo method, the blood supply to testes was cut by crushing the cord with the help of baby burdizzo castrator. In the third method of removal of testes, about 5 ml of novocain (2 per cent) was injected into the cord. The testicles were exposed through the incision made on the posterior aspect of the scrotum and then removed after ligating the cord.

All kids were kept in flock and fed as per the schedule in Table 1. The kids were weighed every 15 days. The animals were slaughtered at 15 kg body weight and dressed by standard practice. After removing the abdominal and thoracic viscera, the carcass was frozen at –20°C in deep freezer for about 48 hr. It was then taken out and cut into seven parts[8].

Sensory evaluation of meat was conducted by a taste panel composed of 9 persons representing different segments of the institute community. Roasts were cooked in an oven at 163°C until their internal temperature reached 78°C. Two 1.3 cm cores of the *Longissimus* muscle of the loin were evaluated for tenderness, flavour

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TABLE 1. SCHEDULE OF FEEDING

Animal wt. (kg)	Feed ingredients/day			
	Milk (lit.)		Concentrate*	Green fodder**
	Morning	Evening	(kg)	(kg)
2.0	0.20	0.20	0.00	0.0
3.0	0.25	0.25	0.00	0.0
5.0	0.30	0.30	0.50	0.0
6.0	0.30	0.30	0.10	ad libitum
7.0	0.35	0.35	0.15	- do -
8.0	0.30	0.30	0.20	- do -
9.0	0.15	0.15	0.25	- do -
10.0	0.15	0.15	0.35	- do -
12.0	0.10	0.10	0.35	1.5
15.0	0.00	0.00	0.35	1.5
20.0	0.00	0.00	0.35	2.0
25.0	0.00	0.00	0.35	2.0

*Wheat bran, 30; maize/barley, 35; groundnut cake, 25; molasses, 7; mineral mixture (supermendif), 2.5 and common salt, 0.5 per cent.

**Greenfodder included either or mixture of maize, berseem and lucerne.

TABLE 2. MEAN SCORES* FOR TENDERNESS, JUICINESS AND FLAVOUR OF MEAT

Castration/ sterilization method	Age at castration/sterilization (months)	Tenderness	Juiciness	Flavour
Burdizzo	2	7.05(6-8)	6.5 (5-8)	6.73(6-8)
	4	6.80(6-8)	6.84(6-8)	6.20(5-8)
	6	7. 4(6-8)	6.83(6-8)	6.46(5-8)
Removal of tests	2	7.30(5-9)	5.9 (5-8)	6.61(5-9)
	4	6.94(6-8)	6.51(6-9)	7.09(6-9)
	6	6.70(5-8)	6.30(5-8)	6.66(4-8)
Vasectomy	2	7.44(5-8)	6.22(5-7)	6.27(6-8)
	4	7.11(6-8)	6.56(4-8)	6.67(6-8)
	6	7.40(6-8)	6.90(6-8)	6.90(6-8)
Intact (Control)		7.45(6-8)	7.00(6-8)	7.19(5-8)

* Values are mean of 18 evaluations.

Values in parenthesis indicate the variability in the scores. Scores obtained for tenderness, flavour and juiciness for intact goats were not statistically different from those obtained for goats vasectomized/castrated at the age of 2,4 or 6 months.

and juciniess on a 9-point hedonic scale ranging from 'like extremely' to 'dislike extremely'.

From each cut, samples of 10, 5 and 2 of lean meat were taken for the determination of moisture, fat and crude protein respectively. Protein was estimated by micro-Kjeldahl method[9], and fat was extracted with petroleum ether for 14-16 hr in Soxhlet extractor.

The method of Saunders *et al*[10] with some modifications was employed for hydrolysis *in vitro* of meat protein. Fresh meat samples (1g) cut into small pieces were defatted with petroleum ether, homogenized in citrate buffer (pH. 2.5) and incubated with 20 mg pepsin at 37°C for 18 hr. The release of free amino nitrogen was estimated by the method of Rosen[11].

For studying hydrolysis *in vitro* with trypsin, the above hydrolysate was heated in boiling water bath for 15 min to inactivate pepsin, pH adjusted to 8.0 with sodium hydroxide and incubated with equal volume of citrate buffer (pH 8.0) and 20 mg trypsin for 18 hr at at 37°C. An aliquot of the hydrolysate was then deproteinized with trichloroacetic acid (10 per cent) and analyzed for amino nitrogen.

The data on the organoleptic properties of meat were subjected to analysis of variance by nested classification. The significance of differences between control and treatments for moisture, ash, crude protein and fat contents and *in vitro* digestibility was tested by Student's 't' test[12].

Results and Discussion

The mean scores for organoleptic qualities of meat are given in Table 2. *Longissimus* muscle of intact goats received a tenderness rating of 7.45, which corresponds to a panel evaluation between 'like very much' and 'like moderately'. The analysis of variance showed that the tenderness scores were not different for intact goats and goats sterilized/castrated by different methods at different ages. Similarly, Baillargeon *et al.*[13] also observed no significant difference between intact lambs and those sterilized or castrated (by burdizzo or surgical removal of testes) at different ages for tenderness.

The juiciness and flavour ratings for *Longissimus* muscle of intact goats were 7.00 and 7.19, respectively, which correspond to panel evaluations of 'like moderately'. The scores received by castrated and vasectomized goats were slightly less and were between panel evaluations of 'like moderately' and 'like slightly'. The differences were, however, not statistically significant. Baillargeon *et al*[13]. and Jourbert[14] observed no marked

TABLE 3. ANALYSIS FOR MOISTURE, PROTEIN AND FAT CONTENTS OF MUSCLES OF INTACT AND CASTRATED/STERILIZED MALE GOATS

Castration/ sterilization method	Age at castration/ sterilization (months)	Moisture (%)	't' values	Protein (%)	't' values	Moisture/Protein ratio	't' values	Fat (%)	't' values
Burdizzo	2	75.7±0.4[a]	0.82	19.6±0.8	2.88**	3.87±0.07	2.26*	2.6±0.3 (1.9)[b]	3.56**
	4	76.7±0.2	0.39	20.6±0.4	1.41	3.72±0.09	1.18	1.3±0.1 (0.9)	0.13
Removal of testes	2	76.2±0.8	0.19	20.7±0.6	1.12	3.69±0.13	1.33	1.6±0.1 (1.0)	1.23
	6	73.2±0.8	2.95**	21.8±0.3	0.14	3.36±0.07	1.26	2.5±0.2 (3.5)	3.69**
Vasectomy	2	75.5±0.7	0.86	20.6±0.5	1.36	3.67±0.11	0.90	2.4±0.3 (3.7)	3.22**
	4	75.4±0.8	0.94	20.0±0.6	1.90*	3.78±0.18	1.18	1.9±0.1 (2.2)	2.58**
	6	75.9±0.2	0.95	20.1±0.4	1.98*	3.78±0.09	1.52	2.5±0.2 (1.9)	4.34**
Intact control		76.4±0.7		21.7±0.6		3.53±0.13		1.3±0.2 (0.7)	

[a] Values are mean±SEM of 14 determinations.

[b] Values given in parenthesis are for the content of *longissimus* muscle obtained from loin cut.

* Statistically significant from control ($P<0.05$); ** $P<0.01$

difference in the flavour of meat between uncastrated and those castrated or sterilized at differenct ages.

Moisture, crude protein and fat contents of meat: The moisture, crude protein and fat contents of the meat were analyzed in goats vasectomized at the age of 2, 4 or 6 months and in those castrated by burdizzo at the age of 2 or 4 months, and by removal of tests at the age of 2 or 6 months. The moisture of meat of intact goats was 76.4 per cent (Table 3), which decreased in goats whose testes were removed at the age of 6 months, while it did not change significantly in goats vasectomized at the age of 2,4 or 6 months, castrated by burdizzo at the age of 2 or 4 months or castrated by removal of the testes at the age of 2 months. Bailargeon *et al*[13], on the other hand, observed that meat from intact lambs contained more water than that from those castrated by burdizzo. The moisture content in the meat from sheep, pigs and rabbits castrated by surgical removal of the testes was also lower than that from their intact conterparts[15].

The protein content (Table 3) in the meat from intact goats was slightly higher than in that from those vasectomized at different ages. The meat from goats castrated by burdizzo at the age of 2 or 4 months, and those castrated by removal of the testes at the age of 2 months also had slightly lower levels of protein. The diminution was statistically significant only in the cases of goats vasectomized at the age of 4 or 6 months, and in those castrated by burdizzo at the age of 2 months. Similarly, a slightly lower level of protein than in intact animals has been reported in sheep[13], pigs[15] and rabbits[15] castrated by removal of testes or by stopping blood supply to the testes.

Moisture to protein ratio was calculated in order to see its corrleation with juiciness/tenderness of meat. Except in case of animals castrated by burdizzo at the age of 2 months, the differences in moisture to protein ratios were not significant between intact goats and those vasectomized or castrated by different methods at different ages.

The intramuscular fat (marbling) in goats left intact (1.34 per cent) was less than in those vasectomized or castrated by burdizzo or removal of testes (Table 3). The increase in intramuscular fat was statistically significant in goats vasectomized at either 2, 4 or 6 months, and in those castrated by burdizzo at 2 months, or by removal of tests at 6 months. Increase in intarmuscular fat as a result of castration has also been reported in cattle[1,15–18].

Marbling has long been considered a mark of quality

TABLE 4. *IN VITRO* DIGESTIBILITY OF MEAT-PROTEIN OF INTACT AND CASTRATED/STERILIZED MALE GOATS

Castration/ sterilization method	Age at castration/sterilization (months)	Pepsin digestion (%)	Pepsin digestion 't' values	Trypsin digestion (%)	Trypsin digestion 't' values
Burdizzo	2	26.2±1.2[a]	4.538**	46.3±3.0	3.529**
	4	28.4±2.8	3.482**	49.9±5.2	2.894*
Removal of tests	2	22.5±4.3	1.198	37.1±4.2	0.643
	6	21.3±3.3	1.236	37.1±3.7	0.712
Vasectomy	2	19.5±2.4	0.934	33.2±4.8	0.199
	4	23.3±2.3	2.275*	41.8±3.5	2.055
	6	16.5±2.7	0.061	37.1±3.7	0.684
Intact (control)		16.7±1.7		34.2±1.6	

[a] Values are mean±SEM of 14 observations

** The values are significantly different from control at $P<0.01$ and at * $P<0.05$.

in meat. Wierbicki *et al*[1]. and Bailey *et al*[17]. reported steers to be more tender and possess more intramuscular fat than bulls. Our results, however, show no such correlation. The differences were not significant among treatments and age at treatments for tenderness, flavour and juiciness of meat, though intramuscular fat content of loin muscles (Table 3) was more in castrated/sterilized goats. Wierbicki *et al*[1]. and Oliver *et al*[5]. also could not find any correlation between marbling and tenderness in cattle and lambs, though steers and wether lambs were more tender and had more intramuscular fat than bulls and intact lambs, respectively.

In vitro digestibility with pepsin and trypsin of meat-protein was studied in goats vasectomized at the age of 2, 4 or 6 months, and in those castrated at the age of 2 or 4 months by burdizzo, and at the age of 2 or 6 months by surgical removal of testes (Table 4). Digestibility of meat-protein was significantly higher in goats castrated by burdizzo than in intact goats; the difference between the age at which the goats were castrated was, however, not significant. The lower digestibility of meat protein in intact goats than in those castrated by burdizzo might be due to more connective tissue in the former. Wierbicki *et al*[1]. reported the beef from bulls to have more connective tissues than that from steers.

No significant difference in digestibility was observed between intact goats and those castrated by removal of testes at the age of 2 or 6 months. Though digestion with pepsin of meat protein was higher in goats vasectomized at the age of 4 months, the difference in overall perpsin-trypsin digestion was, however, not significant between intact goats and those vasectomized at the age of 2, 4 or 6 months.

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NONSTARCHY POLYSACCHARIDES OF ITALIAN MILLET

Nonstarchy polysaccharides (NSP) content in 14 varieties of Italian millet ranged from 1.22 to 2.23% of the dehulled grain. Water-soluble and alkali-soluble NSPs of the 2 varities of the millet were isolated and partially characterized. The yield of alkali-soluble NSP is approximately 6 times the water-soluble NSP. All the NSP preparations contained glucose, galactose, arabinose and xylose. The total carbohydrates in the two varieties were 68 and 82.5% respectively. The ratio of pentoses to hexoses in the NSP preparations was nearly unity.

Italian millet (*Setaria italica*) is grown extensively in India; the grain is used both as food and feed. In India, about 3-24 lakh tonnes of the grain is produced annually. In China this millet is one of the principal food crops and is used in puddings, cereal delicacies and also eaten cooked like rice or in baked products. Nonstarchy polysaccharides (NSP) have an important water-binding function and the content and quality of NSP in a grain influence the baking potential of the grain flour. Though there are reports on the free sugars[1] and starch[2] of Italian millet, there is no published literature on the NSP of this grain. As part of the investigations on the different aspects of Italian millet proteins and carbohydrates, a preliminary characterization of the NSP of this millet has been undertaken and the results are incorporated in this paper.

Italian millet samples were obtained from the Plant Scientist (Millets), University of Agricultural Sciences, Bangalore. The seeds were dehulled using a McGill sheller and ground in a Buhler grinder to pass through a 40 mesh screen

The NSP content of the 14 Italin millet varieties was determined by the method of Fraser *et al*[3]. For isolation of NSP only two varieties, viz., 'RS-118' and 'I. Se-358' were used. Water-soluble NSP were isolated by the method of Lin and Pomeranz[4] and alkali-soluble NSP as described by Karim and Rooney[5]. NSP preparations were hydrolysed in 0.1N H_2SO_4 in sealed glass tubes at 100° for 4 hr. The acid in the hydrolyzates was removed using Dowex-1 resin (HCO_3-form)[5]. The NSP hydrolyzates were chromatographed on Whatman No. 1 filter paper using the solvent systems ethyl acetate: pyridine: water, 8:2:1 (solvent I), ethyl acetate: pyridine: water, 12:5:4 (solvent II), and ethyl acetate: pyridine: water: acetic acid, 12:5:4:1 (solvent III). Sugars were located on the paper using aniline-oxalate reagent[6]. Carbohydrate content of the isolated NSP was estimated after acid hydrolysis by the phenol-sulphuric acid method[7]. For quantitative estimation of sugars, the constitutent sugars were separated using solvent III, the sugars eluted from the chromatogram and estimated colorimetrically by the phenol-sulphuric acid method[7] using appropriate standards.

The NSP content of 14 Italian millet varieties is presented in Table 1 which ranged from 1.22 to 2.23 per

TABLE 1. NONSTARCHY POLYSACCHARIDE CONTENT OF ITALIAN MILLET

Variety	Nonstarchy polysaccharides (% dry wt)
GS-118	1.22
I.Se-709	1.37
SI-76/4	1.43
KHS-1	1.46
SI-80/2	1.48
JNSE-53	1.61
K-221-1	1.61
SI-5307	1.76
I.Se-703	1.78
I.Se-201	1.83
JNSE-26	1.84
I. Se-480	1.85
CO-3	1.95
I.Se-358	2.23

TABLE 2. COMPOSITION OF NONSTARCHY POLYSACCHARIDES (NSP) IN TWO VARIETIES OF ITALIAN MILLET

	RS-118		I.Se-358	
	WSNP	ASNP	WSNP	ASNP
Yield[a]	0.31	1.80	0.48	3.13
Total carbohydrate[b]	67.9	82.5	68.7	78.0
Glucose	21.8	27.0	20.6	28.8
Galactose	12.4	16.9	15.3	8.6
Arabinose	25.5	24.3	23.4	30.4
Xylose	6.8	18.4	11.0	13.9
Pentose: hexose	0.94	0.97	0.96	1.18
Protein[b] (N×6.25)	25.6	4.80	20.6	5.7

[a] g/100 g of dehulled seed.

[b] as per cent of nonstarchy polysaccharide

WSNP – water-soluble nonstarchy polysaccharide

ASNP – alkali-soluble nonstarchy polysaccharide.

cent. The NSP content of Italian millet is higher than that of milled rice[8] which is about 1 per cent, but lower than that of wheat[9] (5.6-9 per cent), sorghum[10] (3.5 per cent) and finger millet[11] (approximately 3.3 per cent).

Table 2 gives the composition of the NSP of 2 varieties of Italian millet. The amount of alkali-soluble NSP is much greater than the amount of water-soluble NSP. The carbohydrate content of Italian millet NSP is comparable to that of preparations from other millets and ranges from 68 to 82.5 per cent. In finger millet, the carbohydrate content of water and alkali-soluble pentosans ranged from 52 to 92 per cent[11], in sorghum 68 to 75 per cent[5], while rice pentosans contain about 80 per cent carbohydrate[8]. The protein contents of Italian millet NSP range from 4.8 to 25.6 per cent. Such wide variations have also been observed in finger millet pentosans which contain 3.9-18. per cent protein[11] and sorghum pentosans which will have 1.3-10.5 per cent protein[10].

Paper chromatography showed that the sugars present in Italian millet NSP are glucose, galactose, arabinose and xylose. These are the same sugars reported to be present in other pentosans also[5,8,12]. Mannose and ribose were not detected which are reported in barley pentosans[13,14]. The predominent sugars in the NSP of Italian millet are arabinose and glucose; xylose and galactose are present in lower amounts. The ratio of pentoses to hexoses is approximately 1 in the NSP of this grain. In other cereals, the ratio of pentoses to hexoses vary widely[5,8,11,12]. In this respect the NSP of Italian millet are different from those of other cereals and millets.

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COMPARATIVE ANTIFUNGAL ACTIVITY OF CAMPHOR, BAVISTIN AND DICLORAN

The *in vitro* antifungal activity of camphor, Bavistin and Dicloran, against the mycelial growth of *Rhizopus stolonifer*, *Aspergillus niger*, *Penicillium digitatum*, *Alternaria sp* and *Fusarium oxysporum* was studied. Camphor exhibited antifungal activity on all the 5 test fungi and the inhibition varied from 40 to 76% depending on the test fungi. Bavistin at 100 ppm caused 100% inhibition of mycelial growth of 3 pathogens, but partially on *R. stolonifer* and *Alternaria sp* at 1000 ppm. Dicloran at 100 ppm was more effective than Bavistin, as it brought about 100% inhibition against 4 of the test pathogens, with the exception of *F. oxysporum* for which 1000 ppm of Dicloran was needed. Further, in an atmosphere of camphor vapours, sporulation of all the 5 test fungi was inhibited.

Camphor is one of the naturally occurring substances widely used in pharmaceutical formulations[1]. In our country, camphor is also used in small amounts in *Pans* and preparation of sweets etc. for the characteristic odour. Maruzzella and Balter have observed the presence

of antifungal activity in camphor[2]. However, its antifungal activity was not compared to that of any standard synthetic fungicide.

The present study reports the relative efficacy of commercially available white solid camphor (1, 7, 7-Trimethyl bicyclo (2.2.1) heptan-2-one) and that of two synthetic fungicides viz. Bavistin (2-(Methoxy-carbamoyal)-benzimidazole) and Dicloran (2, 6-Dichloro-4-nitroaniline) in controlling *Rhizopus stolonifer*, *Aspergillus niger*, *Pencillium digitatum*, *Alternaria sp. and Fusarium oxysporum* which cause spoilage of fruits and vegetables.

Inoculum of all the fungi was prepared by aseptic addition and even spreading of 1 ml of spore suspension (50,000 spores/ml) on 20 ml of solidified potato dextrose agar (PDA) medium contained in 90 mm petriplates, incubating at 28°C and the mycelium before sporulation was used for inoculation.

Camphor, dissolved in distilled ethanol (1g in 10 ml) was added to 100 ml of melted PDA medium to 100, 500, and 1000 ppm concentration. Medium without camphor served as control: 20 ml lots of the medium were poured into 90 mm petriplates, allowed to solidify, inoculated at the center with a 7 mm inoculum plug of the test fungi separately and incubated at 28°C for 3 days. Five replicates were maintained for each treatment. Diameter of the fungal colony was measured and the percentage inhibition was calculated using the equation given by Vincent[3] as:

$$I = \frac{(C - T)}{C} \times 100$$

Where, I =per cent inhibition
C=colony diameter of the control
and T =colony diameter of the tested plates.

Similarly, the two standard fungicides viz. Bavistin and Dicloran, were tried at 100, 500 and 1000 ppm concentrations.

Camphor displayed wide spectrum of antifungal activity (Table 1). At 1000 ppm, it exerted the minimum

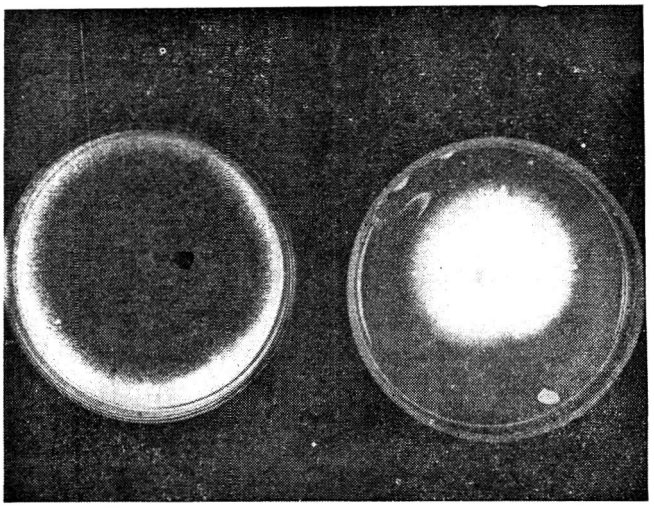

Fig. 1. Effect of Camphor on sporulation of *A. niger*. Left—control; Right—treated

of 40 per cent inhibition on *A. niger* and on *F. oxysporum*, and 76.4 per cent, the maximum among all the test organisms. Bavistin at 100 ppm brought about complete inhibition in the growth of *A. niger*, *P. digitatum* and *F. oxysporum*, whereas 1000 ppm of it could cause 83.2 per cent inhibition of *R. stolonifer* and 43.8 per cent of *Alternaria sp.* Dicloran at 100 ppm was more effective than Bavistin. as it brought about complete inhibition of mycelial growth in all the test fungi, except in *F. oxysporum* on which 1000 ppm of the fungicide was needed to bring 100 per cent inhibition.

Further, it was also observed that, camphor (0.1g) when kept in petriplates (90 mm) containing medium inoculated with test fungi, inhibited sporulation and a typical case of *A. niger* is shown in Fig. 1. This property of inhibition of sporulation may be of practical help in preventing the spread of the pathogens.

In conclusion, it can be stated that although camphor, exhibits wide spectrum antifungal activity, it is required

TABLE 1. INHIBITION OF FUNGAL GROWTH BY CAMPHOR, BAVISTIN AND DICLORAN

Test fungi	Camphor (ppm)			Bavistin (ppm)			Dicloran (ppm)		
	100	500	1000	100	500	1000	100	500	1000
*R. stolonifer**	4.5	43.2	75.2	12.3	35.5	83.2	100.0	100.0	100.0
A. niger	0.0	16.7	40.0	100.0	100.0	100.0	100.0	100.0	100.0
P. digitatum	0.0	20.0	50.0	100.0	100.0	100.0	100.0	100.0	100.0
Alternaria sp	0.0	25.4	54.2	10.2	33.8	45.8	100.0	100.0	100.0
F. oxysporum	0.0	34.3	76.4	100.0	100.0	100	74.5	80.4	100.0

* Observation made after 24 h of incubation.

at much higher concentration for complete inhibition of fungal growth than Bavistin and Dicloran, the two synthetic fungicices.

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SPECTROPHOTOMETRIC METHOD OF DETERMINATION OF CARBOFURAN RESIDUES

A method has been developed for the determination of carbofuran (2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl methyl carbamate) residues based on its hydrolysis with methanolic potassium hydroxide to carbofuran phenol, which on reaction with 4-aminoantipyrine in the presence of an alkaline oxidizing agent produces an orange colour dye with an absorption maximum at 475 mm. The relationship between absorbance and concentration of carbofuran is linear in the range of 0.5 to 20 μg/ml. This method can detect as low as 0.3 ppm of carbofuran in food grains.

Carbofuran (2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl methyl carbamate) is a broad spectrum insecticide and nematocide with both contact and systemic action. Several gas chromatographic methods[1-4] and spectrophotometric methods[5-7] have been developed for the determination of carbofuran residues from agricultural crops and grains. The gas chromatographic methods mentioned above can detect carbofuran residues from 0.01 to 0.2 ppm level and the spectrophotometric method of Rangaswamy *et al*[6] can detect upto 0.25 ppm of carbofuran in crops. Although gas chromatographic methods are ideal for determining and estimating pesticides, their use is often limited by nonavailability and high cost. The nonavailability of other chromogenic reagents at times, has necessitated a search for an alternative reagent. This method is based on the reaction between 4-aminoantipyrine and phenols to give coloured products[8]. As the method described below is not as sensitive as the method which specifies the use of aniline as a chromogenic agent[6], it would serve as an alternate method for the determination of carbofuran residues in grains.

Preparation of standard curve: Aliquots of carbofuran working standard solution containing 0, 25, 50, 100, 200, 400, 600, 800 and 1000 μg of carbofuran were transferred to clean dry separatory funnels. This was followed by the addition of 2.5 ml of 0.2 N methanolic potassium hydroxide and the solution was shaken for 5 min intermittently. The solution was neutralized with 0.2 N hydrochloric acid and adjusted to 9-10 pH with dilute ammonium hydroxide (diluted 1 part of ammonium hydroxide (25 per cent NH_3, 0.91 sp. gr) with 2 parts of water). At this point 2 ml of 3 per cent 4-aminoantipyrine and 2 ml of 20 per cent potassium ferricyanide solutions in water were added to the separatory funnel. An orange colour with an absorption maximum at 475 nm was formed immediately which was extracted 3 times with 10 ml portions of chloroform. The chloroform layer was passed through anhydrous sodium sulphate in a funnel to remove traces of moisture and the resulting chloroform solution was transferred to a 50 ml volumetric flask. The colour adsorbed on sodium sulphate was washed down twice with 5 ml portions of chloroform and the volume was made upto mark with chloroform. Absorbance was recorded at 475 nm against a blank similarly prepared without carbofuran. Absorbance was plotted against μg of carbofuran/ml reaction mixture to get the standard curve.

Recovery of carbofuran from spiked grains and pulses: Hundred grams each of cereal grains (rice, wheat, jowar) and pulses (pigeon pea, blackgram, green gram) were spiked with 5 ml of methanolic solution containing 30 60, 120 and 240 μg of carbofuran in a blender. Samples were extracted by the clean up procedure of Cook *et al*[3]. The final residue after evaporation of the extract obtained by the above procedure was redissolved in 5 ml methanol and colour development was done as outlined in standard curve preparation. The absorbance was recorded against a crop control (blank).

The linear relationship between the absorbance at 475 nm and the concentration of carbofuran is valid upto 20 μg/ml reaction mixture. The orange colour formed immediately by the reaction of 4-aminoantipyrine with carbofuran phenol is stable upto 8 hr. However, the absorbance should be recorded within 1 hr after developing colour as there is cloud formation.

To check the efficiency of the method described, grains (rice, wheat, jowar) and pulses (pigeon pea, black gram, green gram) were spiked with 30-240 μg carbofuran and recovery studies were conducted. Recoveries ranged from 94.58-99.66 per cent (Table 1). Natural pigments

TABLE 1. RECOVERY OF CARBOFURAN FROM CEREAL GRAINS AND PULSES

Sample	Carbofuran		Recovery (%)
	Added (μg)	Detected (μg)[a]	
Rice	30.0	29.8 ± 0.71	98.33
	60.0	58.9 ± 0.35	98.16
	120.0	115.0 ± 0.59	95.83
	240.0	230.0 ± 3.10	95.83
Wheat	30.0	29.7 ± 0.39	99.00
	60.0	59.0 ± 0.84	98.33
	120.0	117.3 ± 1.21	97.75
	240.0	230.0 ± 1.48	95.87
Jowar	30.0	29.0 ± 1.10	96.66
	60.0	59.3 ± 0.67	98.83
	120.0	116.2 ± 0.98	96.83
	240.0	234.5 ± 4.30	97.71
Pigeon pea	30.0	29.9 ± 0.11	99.66
	60.0	57.1 ± 0.31	95.16
	120.0	115.0 ± 0.59	95.83
	240.0	229.7 ± 0.40	95.71
Black gram	30.0	28.9 ± 0.75	96.30
	60.0	56.8 ± 0.65	94.66
	120.0	114.5 ± 1.85	95.41
	240.	227.0 ± 0.81	94.58
Green gram	30.0	29.1 ± 0.85	97.00
	60.0	58.5 ± 1.26	97.50
	120.0	116.0 ± 1.66	96.66
	240.0	230.0 ± 1.33	95.83

[a] Mean of 8 analyses ± Standard deviation.

and free phenols present in the seeds did not interfere in the estimation as evidenced from clear crop control solution (blank).

The proposed spectrophotometric method is more sensitive than other spectrophotometric methods[5,7] with reported sensitivities of 2 to 0.5 μg. Although the present method is not as sensitive as the method which specifies aniline as a chromogenic agent[6] with a sensitivity of 0.25 ppm, it would serve as an alternate method for the determination of carbofuran residues from grains (detection limit 0.3 ppm.),

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EFFECT OF COOKING AND STORAGE ON CHOLESTEROL CONTENT OF QUAIL MEAT

The effect of cooking and storage on the cholesterol content of quail muscles was studied. In fresh thigh and breast muscles, cholesterol contents were 1.04 mg and 1.18 mg per gram respectively. Seventy per cent of total cholesterol was present in the free form and the rest in esterfied form. The cholesterol contents in the thigh and breast muscles decreased on cooking.

Variations in cholesterol contents in different types of meat due to cooking have been reported; cooking decreased the cholesterol content in chicken[1], but not in turkey[2]. Information on cholesterol content of quail meat and changes due to cooking and storage is not available. This study was conducted to determine the relative amounts of cholesterol and its ester in quail muscles and associated changes in their contents due to cooking and storage.

Seventy eight quails (*Coturnix coturnix japonica*) were dressed. Thirty nine quails were cooked at 1.1

kg per cm^2 pressure for 8 min. Raw and cooked quails were packed in polyethylene bags of 150 guage and stored at 4 and -10°C. Samples from thigh and breast muscles were taken in duplicate for estimation of cholesterol on 3, 6 and 9 days of storage at 4°C and 30, 60 and 90 days of storage at -10°C. Lipid from muscles was extracted[3]. Unidimensional thin layer chromatography using triple solvent system was used for separation of free and esterified cholesterol[4]. Free (FC) and esterified cholesterol (EC) were eluted from silica gel with chloroform and estimated[5].

The mean values for cholesterol contents of thigh and breast muscles are presented in Table 1. The values obtained in the present study are higher than those reported for broiler by earlier workers[6-8]. There is, however, no information on cholesterol content of quail meat. Turk and Barnet[9] reported that quail eggs contained more cholesterol than chicken eggs. Hence, the higher cholesterol contents in quail than those in chicken meat may be due to species difference. Seventy per cent of total cholesterol is present in the free form and remaining 30 per cent in the esterified form. A comparison of means revealed that both fractions of cholesterol decreased due to cooking, but free cholesterol decreased significantly ($P < 0.05$). A decrease in cholesterol content due to cooking was also reported in chicken[10]. The decrease in cholesterol content on cooking was ascribed to the denaturation of protein which altered protein-cholesterol complex. No significant change was observed in contents of free cholesterol (FC) and esterified cholesterol (EC) fractions in cooked muscles stored at 4°C. In raw thigh muscle, however, a significant decrease in EC was observed on storage. Storage at -10°C caused a significant increase in FC content of raw breast muscle ($b=0.005\pm0.001$) and a significant decrease in FC ($b= -0.003\pm0.001$) and EC ($b= -0.0004\pm0.0001$) contents of cooked breast muscle. Inconsistent changes in cholesterol content due to cooking and freezing were also noted by other workers. A drop in cholesterol content was observed after cooking in chicken[1], but this was not true in case of turkey[2]. Freezing increased the cholesterol content of oyester and shrimp meat, but did not affect its level in crab meat[11]. Changes observed in the cholesterol content of quail meat due to cooking and storage has not been fully understood. Further investigation would be needed to find out the physico-chemical changes occurring in the protein-cholesterol complexes of the tissues after cooking and freezing.

TABLE 1. CHOLESTEROL CONTENTS* IN THIGH AND BREAST MUSCLES OF QUAIL

Storage period (days)	Raw thigh muscle			Cooked thigh muscle			Raw breast muscle			Cooked breast muscle		
	Total	FC	EC	Total	FC	EC	Total	FC	EC	Total	FC	EC
Stored at 4°C												
0	1.04 ±0.04	0.72 ±0.03	0.32 ±0.26	0.72 ±0.16	0.64 ±0.16	0.08 ±0.01	1.18 ±0.01	1.07 ±0.02	0.11 ±0.01	0.95 ±0.05	0.83 ±0.05	0.12 ±0.01
3	1.03 ±0.02	0.91 ±0.04	0.12 ±0.02	1.06 ±0.07	0.90 ±0.10	0.16 ±0.04	0.90 ±0.08	0.80 ±0.07	0.10 ±0.01	0.79 ±0.14	0.71 ±0.14	0.08 ±0.01
6	1.33 ±0.09	1.23 ±0.09	0.10 ±0.01	1.12 ±0.28	1.04 ±0.28	0.08 ±0.01	1.47 ±0.21	1.34 ±0.19	0.13 ±0.01	0.77 ±0.01	0.69 ±0.01	0.08 ±0.01
9	1.42 ±0.27	1.28 ±0.23	0.14 ±0.04	0.81 ±0.04	0.71 ±0.04	0.10 ±0.01	0.70 ±0.02	0.63 ±0.03	0.07 ±0.01	0.77 ±0.13	0.68 ±0.11	0.09 ±0.01
Stored at - 10°C												
30	1.16 ±0.15	1.05 ±0.17	0.11 ±0.02	0.93 ±0.16	0.83 ±0.11	0.10 ±0.01	0.82 ±0.01	0.66 ±0.02	0.16 ±0.02	0.74 ±0.08	0.65 ±0.07	0.90 ±0.01
60	1.13 ±0.03	0.96 ±0.03	0.17 ±0.06	0.78 ±0.11	0.68 ±0.09	0.10 ±0.02	0.65 ±0.01	0.58 ±0.01	0.07 ±0.01	0.69 ±0.03	0.60 ±0.03	0.08 ±0.01
90	1.13 ±0.03	0.96 ±0.03	0.17 ±0.08	0.77 ±0.11	0.67 ±0.09	0.10 ±0.02	0.64 ±0.01	0.57 ±0.01	0.07 ±0.01	0.69 ±0.03	0.60 ±0.03	0.09 ±0.01

* mg per g of wet tissue, FC = Free cholesterol, EC = Esterified cholesterol.

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ESSENTIAL AMINO ACID CONTENT OF BUFFALO MEAT

The essential amino acid composition of buffalo meat has been determined by microbiological assay. Chemical scores calculated for individual amino acids showed that aromatic amino acids, phenylalanine and tyrosine are the first limiting amino acids followed by isoleucine and tryptophan. Essential amino acid index, biological value and nutritional index have been calculated using amino acid composition data.

Buffalo meat constitutes a major portion of the meat export from India which is about 8300 tonnes per annum[1]. With increasing cost of mutton and other types of meat, buffalo meat is gaining importance. There is very little information available on the amino acid composition of buffalo meat. The present communication deals with the essential amino acid content of buffalo meat from which the essential amino acid index[2], biological value[3] and nutritional index[4] have been computed.

Buffalo meat samples collected from a local butcher were used in these experiments. Meat chunks were seperated from bones and visible fat, minced and used for the studies. The essential amino acid analysis was carried out by microbiological assay technique as per the procedure of Barton-Wright[5]. The defatted material was hydrolysed with 2.5N hydrochloric acid under vacuum for 18 hr at 110°C. Alkaline hydrolysis was carried out using 5N sodium hydroxide. Lysine, methionine, phenylalanine, histidine and cystine were estimated using *Leuconostoc mesenteroides* P-60 as test organism. Leucine, isoleucine, valine and tryptophan were estimated using *Lactobacillus arabinosus*. Arginine and threonine were estimated using *Streptococcus faecalis*.

The essential amino acid composition and chemical scores of individual amino acids are presented in Tables 1 and 2 respectively. Two samples with two replicates were selected for the study. Average of the replicates is presented in the tables. Values given for tyrosine and cystine are taken into account for calculating the chemical score. The data presented in Table 1 show that lysine is present in high concentration and the other essential amino acids are in adequate quantities. The

TABLE 1. ESSENTIAL AMINO ACID COMPOSITION OF BUFFALO MEAT AND EGG PROTEIN

Amino acid	Buffalo meat (Amino acid g/100 g protein)	Hen's egg[3]
Arginine*	6.5	
Histidine*	3.1	
Lysine	8.9	6.4
Tryptophan	1.2	1.6
Phenylalanine	3.6	5.8
Tyrosine	3.2	4.2
Methionine	3.2	3.1
Cystine	1.6	2.4
Threonine	4.0	5.1
Leucine	7.8	8.8
Isoleucine	4.7	6.6
Valine	5.9	7.3
Total essential amino acids	44.10	51.30

*Not included for calculating total essential amino acids.

TABLE 2. CHEMICAL SCORES FOR INDIVIDUAL AMINO ACIDS IN BUFFALO MEAT

Amino acid	Chemical score
Lysine	162
Leucine	103
Methionine & Cystine	98
Valine	95
Threonine	91
Tryptophan	87
Isoleucine	83
Phenylalanine + tyrosine	79

Chemical score for buffalo meat is 79.

chemical score for buffalo meat is 79, the aromatic amino acids phenylalanine and tyrosine are the first limiting amino acids followed by isoleucine and tryptophan (Table 2).

The chemical score, essential amino acid index and biological value for buffalo meat calculated from the amino acid composition data will be 79, 89 and 85 respectively as compared to 69, 88 and 76 for beef muscle proteins[6]. The nutritional index for buffalo meat is 75.

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CHANGES IN QUALITY AND ACCEPTABILITY OF REFRIGERATED QUAIL (*COTURNIX COTURNIX JAPONICA*) EGGS STORED AT ROOM TEMPERATURE

Significant increase in loss in egg weight, aircell diameter and % yolk and decrease in albumen index, yolk index, % whole albumen and overall acceptance scores were observed, when quail eggs, first held under refrigeration (temperature, 5.0+1°C; R. H., 80-85%) for 60 days, were subsequently held at room temperature, 28.8-32.7°C; R.H., 65.7-88.8%) for 5 days.

Eggs are susceptible to quality changes during storage. Marked deterioration has been observed in chicken eggs within 3 days regardless of temperature[1] and the egg contents deteriorate in physical attributes like albumen and yolk indices and increase in diameter and depth of aircell during storage[2]. No published literature is available on this aspect on quail eggs and hence the present study was undertaken to find out the extent of changes.

Three hundred seventy fresh quail eggs having an average weight of 9.89 g were utilized for the present study; 10 eggs were used as control and remaining 360 eggs (120 eggs per storage) were subjected to 30, 45 and 60 days of refrigerated storage. Eggs were withdrawn at the end of each refrigeration period and subjected to subsequent room storage for 5 days. Twenty eggs (10 each for physical quality measurement and sensory evaluation) from each refrigerated storage were utilized daily from 0 to 5 days of room storage. Loss in egg weight during storage was calculated by difference in initial and stored egg weight. Shape index was calculated from egg length and breadth. Albumen and yolk indices were calculated as per the methods of Heiman and Carver[3] and Sharp and Powel[4] respectively. Shell thickness was measured with the help of AMES Thickness Measure (No. 25 ME) and aircell diameter was measured by vernier callipers. Weight of whole albumen, yolk and shell with membrane was recorded. pH of various components viz. whole albumen, yolk and whole egg were measured by a pH meter. Consumers' acceptance of stored eggs was compared with fresh eggs by a panel of 4 permanent judges. Room temperatures and relative humidity ranged from 28.8-32.7°C and 65.7-88.8 per cent respectively during storage. Refrigeration temperature and relative humidity were 5.0±1°C and 80-85 per cent respectively. The data were analysed statistically as per the methods of Snedecor and Cochran[5] and differences for significance were tested by multiple range test[6].

A stepwise significant increase in per cent loss in egg weight during refrigeration and maximum loss on 4th day of subsequent room storage was observed and it was found to decrease on 5th day (Table 1). The rate of

TABLE 1. LOSS IN EGG WEIGHT DURING STORAGE

Refrigeration storage (days)	Room storage (days) 0	1	2	3	4	5	Mean ± SE
30	4.13 ±0.16	5.01 ±0.42	5.12 ±0.24	5.94 ±0.36	6.44 ±0.42	6.59 ±0.36	5.54^{A} ±0.37
45	5.60 ±0.36	6.13 ±0.43	7.78 ±0.42	9.26 ±0.73	10.02 ±0.67	9.09 ±0.55	7.98^{B} ±0.59
60	7.57 ±0.65	8.80 ±0.52	9.49 ±1.33	10.95 ±0.99	11.67 ±1.18	11.92 ±1.34	10.07^{C} ±1.15
Mean ± SE	5.77^{a} ±0.44	6.65^{ab} ±0.46	7.46^{b} ±0.82	8.72^{c} ±0.74	9.38^{c} ±0.82	9.20^{c} ±0.86	—

Values with similar small letters in rows and capital letters in column are not significantly ($P<0.05$) different.

TABLE 2. CHANGES IN INTERNAL QUALITY AND EGG COMPONENTS (% BY WEIGHT)

Refrigeration storage (days)	Room storage (days) 0	1	2	3	4	5	Mean ± SE
			Albumen index*				
30	0.10	0.08	0.09	0.06	0.06	0.06	0.08^{A}
45	0.08	0.08	0.08	0.06	0.05	0.06	0.07^{A}
60	0.09	0.09	0.07	0.07	0.07	0.05	0.07^{A}
Mean	0.09^{a}	0.08^{a}	0.08^{a}	0.06^{b}	0.06^{b}	0.06^{b}	—
			Yolk index				
30	0.45 ±0.01	0.35 ±0.01	0.31 ±0.01	0.25 ±0.00	0.23 ±0.00	0.21 ±0.01	0.30^{A} ±0.01
45	0.42 ±0.00	0.36 ±0.00	0.29 ±0.01	0.25 ±0.01	0.23 ±0.00	0.24 ±0.00	0.30^{A} ±0.01
60	0.40 ±0.00	0.35 ±0.00	0.31 ±0.00	0.27 ±0.00	0.24 ±0.00	0.20 ±0.00	0.30^{A} ±0.00
Mean ± SE	0.42^{a} ±0.01	0.35^{b} ±0.01	0.30^{c} ±0.01	0.26^{d} ±0.01	0.23^{e} ±0.00	0.22^{e} ±0.00	—
			Aircell diameter (cm)				
30	1.59 ±0.01	1.58 ±0.04	1.61 ±0.02	1.71 ±0.03	1.68 ±0.03	1.64 ±0.03	1.64^{A} ±0.03
45	1.70 ±0.02	1.72 ±0.07	1.68 ±0.03	1.83 ±0.03	1.80 ±0.05	1.78 ±0.03	1.75^{B} ±0.05
60	1.72 ±0.05	1.72 ±0.03	1.83 ±0.08	1.89 ±0.06	1.73 ±0.03	1.89 ±0.06	1.80^{B} ±0.06
Mean ± SE	1.67^{a} ±0.03	1.67^{a} ±0.05	1.70^{ab} ±0.05	1.81^{c} ±0.04	1.74^{ab} ±0.04	1.77^{bc} ±0.04	—

Table 2 continued on next page

Table 2. Continued

Refrigeration storage (days)	Room storage (days) 0	1	2	3	4	5	Mean±SE
			% Whole albumen				
30	53.31 ±1.20	47.28 ±1.22	46.25 ±1.91	47.89 ±1.25	46.57 ±1.27	49.90 ±0.73	48.53^{A} ±1.31
45	51.33 ±1.12	49.74 ±1.24	44.76 ±1.40	47.81 ±0.59	48.68 ±1.42	48.36 ±1.38	48.45^{A} ±1.22
60	47.59 ±1.61	45.64 ±1.66	49.91 ±0.75	46.83 ±0.79	43.05 ±1.89	45.57 ±1.24	46.43^{B} ±1.39
Mean±SE	50.74^{a} ±1.33	47.55^{b} ±1.39	46.97^{b} ±1.44	47.51^{b} ±0.92	46.10^{b} ±1.55	47.94^{b} ±1.15	—
			% Yolk				
30	33.37 ±0.83	38.87 ±1.21	40.16 ±1.60	38.60 ±1.12	39.93 ±1.19	37.65 ±0.53	38.10^{A} ±1.13
45	35.69 ±1.05	37.32 ±1.14	41.50 ±1.47	39.73 ±0.62	39.04 ±1.35	39.76 ±1.21	38.84^{A} ±1.17
60	39.49 ±1.46	42.29 ±1.57	37.68 ±0.80	39.83 ±0.76	43.80 ±1.45	41.91 ±0.95	40.83^{B} ±1.21
Mean±SE	36.18^{a} ±1.14	39.49^{b} ±1.32	39.78^{b} ±1.34	39.39^{b} ±0.86	40.92^{b} ±1.33	39.77^{b} ±0.94	—

Albumen index, yolk index, aircell diameter (cm), % whole albumen and yolk by weight in fresh quail egg were 0.11,0.47,0.59, 54.50 and 31.06 respectively.

* S.E. not mentioned being very small (coming to 4th decimal place). Values with similar small letters in rows and capital letters in columns are not significantly ($P<0.05$) different.

weight loss became less towards the end. No significant difference in shape index was found at any storage temperature.

Results on changes in internal quality and egg components by weight are presented in Table 2. No significant change in albumen and yolk indices was observed during refrigerated storage, whereas during subsequent room storage, albumen index reduced on 3rd day and this reduction was constant till 5th day. The decline in albumen quality was due to loss of water and carbondioxide which occurred more rapidly at higher temperatures and resulted in slight alkalinity which caused the long mucin fibres to break[7]. A significant decline in yolk index was observed from 0 to 4th day at room storage which might be due to flattening of yolk during aging. Similar changes in chicken egg yolk during storage were reported by Romanoff and Romanoff[8]. No significant change in shell thickness was found at any storage temperature which indicated that storage has no effect on shell thickness. Significantly maximum increase in aircell diameter was observed at 60 days refrigeration and 3rd day of subsequent room storage which might be due to escape of moisture and gases.

Significant reduction and elevation, in per cent whole albumen and yolk weight respectively were found after 60 days refrigeration and 1st day of room storage. Thinning of albumen in hen's egg was due to breakage of mucin fibres[9] and also due to dissociation of lysozyme-ovamucin complex during storage[10]. The increase in yolk weight can be attributed to the elevation of yolk moisture content due to migration of water from albumen to yolk[11]. No conclusion could be drawn about the changes in shell with membrane weight during storage.

pH of fresh whole albumen, yolk and whole egg was 8.79, 6.12 and 7.28 which increased to 9.85, 7.66 and 8.91

TABLE 3. OVERALL ACCEPTANCE SCORES* OF STORED EGGS

Refrigeration storage (days)	Room storage (days)						Mean ± SE
	0	1	2	3	4	5	
30	6.00 ± 0.00	6.00 ± 0.00	5.75 ± 0.24	5.75 ± 0.24	5.25 ± 0.48	4.75 ± 1.66	5.58^{A} ± 0.72
45	6.00 ± 0.00	5.75 ± 0.24	5.50 ± 0.50	5.75 ± 0.24	5.50 ± 0.28	4.00 ± 0.41	5.42^{A} ± 0.32
60	5.25 ± 0.24	4.00 ± 0.71	3.75 ± 0.63	4.00 ± 0.41	3.25 ± 0.48	3.00 ± 0.41	3.88^{B} ± 0.51
Mean ± SE	5.75^{a} ± 0.14	5.25^{ab} ± 0.44	5.00^{b} ± 0.49	5.17^{ab} ± 0.32	4.67^{b} ± 0.42	3.92^{c} ± 1.01	—

Fresh eggs had overall acceptance score of 7.00.

* 7, like extremely and 1, dislike extremely. Values with similar smaller letters in rows and capital letters in column are not significantly ($P<0.05$) different.

respectively when 60 days refrigerated eggs were stored for 5 days at room temperature. Tiwari and Panda[12] reported a pH of 9.49 of quail whole albumen at 4 weeks of room storage. Increase in alkalinity of albumen causes the loss of its gel like characteristics resulting in low grade egg[13]. Increase in yolk and whole egg pH might be due to escape of more carbon dioxide at higher than at lower temperature[8].

Though overall acceptance score showed a significant reduction at 60 days of refrigeration and 5 days of room storage (Table 3) on initial values, still the eggs were acceptable to the taste panelists.

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STUDIES ON PREPARATION OF BEVERAGE FROM SULPHITED MANGO PULP

Ready-to-serve mango beverage could be prepared from the pulps of 'Totapuri', 'Dusehri', 'Chousa', 'Lucknow' local, 'Langra', 'Desi' and 'Fazli' varieties of mango. The beverage with acceptable organoleptic quality was prepared from preserved pulp by pre-heating and using 600 ppm SO_2, which is within the permissible limits.

Mango pulp forms an important basic material for the preparation of squash, juice, nectar, ready-to-serve (RTS) beverage etc. Adsule and Roy[1], Dhar *et al*[2]. and Roy *et al*[3]. have investigated utilization of north

Indian varieties of mango. The present investigation, was carried out to evaluate some important commercial varieties of north India, marketed in Ludhiana, for making beverages from stored mango pulp.

Different varieties of mango purchased from the local market during 1980 and 1981 seasons were stored at room temperature for ripening. Ripe fruits were washed, stored, trimmed to remove the undesirable portion and pulped in a pulping machine. The pulp was analysed immediately for total soluble solids (TSS), acidity, pH and ascorbic acid[4] content and β-carotene. Organoleptic evaluation of the chilled RTS beverage was carried out by a trained panel of 10 judges using numerical scale, 4=excellent, 3=good, 2=fair, 1=poor for colour, flavour and taste. TSS of the pulp was adjusted to 12 per cent and acidity to 0.6 per cent (as citric acid). The pulp after dilution with water was heated with stirring in a stainless steel steam jacketed kettle, cooled and preserved with 700 ppm SO_2 in 2.5 l capacity bottles.

RTS beverage prepared to contain 15 per cent pulp, 13° Brix and 0.3 per cent acidity was filled hot at 80°C into 200 ml bottles, crown corked, processed for 15 min at 80-85°C, cooled and stored at room temperature. 'Totapuri' mango withstands long distance, when transported in bulk in truck loads to Ludhiana from south India, while other varieties from Uttar Pradesh are transported after packaging in wooden boxes. Large incidence of fruit fly insect damage in 'Totapuri' necessitates slicing, manually to remove the insect damaged portion prior to pulping. TSS contents ranged in 'Chousa', 'Langra,' 'Dusehri' and 'Lucknow local' varieties from 19.5 to 20.0, in 'Totapuri' and 'Desi' from 16.5 to 17.0, and in 'Fazli' 14.3 per cent. 'Desi' mango pulp recorded highest acidity (0.32 per cent). This variety has minimum Brix-acid ratio (53.2) and 'Dusehri' the highest (162.5). Ascorbic acid content was maximum in 'Desi' mango (34 mg/100 g) and in others it ranged from 11 to 34 mg per cent (Table 1). β-carotene was maximum in 'Dusehri' and minimum in 'Langra'

TABLE 1. CHEMICAL COMPOSITION OF PULP OF IMPORTANT VARIETIES OF MANGO

Variety	T.S.S. (°Brix)	pH	Acidity (as % citric acid)	Ascorbic acid (mg/100g)	β-carotene (mg/100g)
Totapuri	16.5	4.2	0.27	13.0	3.5
Dusehri	19.5	4.9	0.12	11.0	9.1
Chousa	20.0	4.7	0.20	23.0	3.9
Lucknow local	20.0	4.4	0.25	15.6	7.0
Langra	19.5	5.0	0.17	25.0	2.1
Desi	17.0	4.0	0.32	34.0	6.6
Fazli	14.5	4.4	0.13	16.0	3.3

TABLE 3. ORGANOLEPTIC SCORE OF MANGO READY-TO-SERVE BEVERAGE

Variety	Colour	Flavour	Taste	Total score
Dusehri	3.5	3.5	3.0	10.0
Langra	3.3	3.1	3.0	9.4
Chousa	2.8	3.0	2.8	8.6
Fazli	2.6	2.6	2.9	8.1
Totapuri	2.4	2.6	2.9	7.9
Desi	2.4	2.4	2.9	7.7
Lucknow local	2.3	2.4	2.9	7.6
Mixed	3.3	3.3	3.1	9.7

TABLE 2. PHYSICAL CHARACTERISTICS OF IMPORTANT VARIETIES OF MANGO

Variety	Fruit wt. (g)	Coring and trimming loss (%)	Pulp recovery (%)	Stones (%)	Peel (%)	Handling loss (%)
Totapuri	330	2.1	65.0	17.5	12.5	2.9
Dusehri	161	6.2	63.8	14.8	12.1	3.1
Chousa	220	3.3	66.0	16.9	9.8	4.0
Lucknow local	151	11.6	58.5	16.0	10.7	3.2
Langra	218	3.8	71.5	12.6	9.1	3.0
Desi	96	4.5	55.8	22.4	13.0	4.3
Fazli	375	2.9	58.0	15.1	20.9	3.1

(Table 1). The yield of stones was maximum in 'Desi' and minimum in 'Langra'. Similar trend was found for the content of peel, also. Loss in handling during pulping was maximum in 'Desi' being 4.3 per cent. 'Langra' variety was rated best on the basis of yield followed by 'Fazli', 'Totapuri', 'Chousa', 'Dusehri', 'Lucknow local' and 'Desi' (Table 2).

The SO_2 content in stored pulp ranged from 590 to 696 ppm with a mean value of 671 ppm. Residual SO_2 in RTS beverage ranged from 58 to 68 ppm with mean value of 64 ppm. This could however be lowered by boiling the juice for 10 min before bottling. Total score for sensory evaluation ranged from 7.7 to 10.0 (Table 3). Score increased to 9.7 when the beverage was prepared from mixed pulp resulting in improved flavour. Cheaper pulp from non-grafted varieties could be blended with the pulps from grafted mangoes to reduce the cost, without affecting the quality of product. The cost of preserved pulp ranged from Rs. 4 to Rs. 6 per kg; pulp from 'Totapuri', being the lowest. Since the blended pulp results in a better product, with respect to sensory characteristics and with lower cost, commercial feasibility of this may be explored.

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REVIEW PAPER

Interconversion of Hexachlorocyclohexane Isomers

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In view of large differences in the toxicity of individual HCH isomers, the phenomenon of their interconversion appears to be quite important, especially so, from the standpoint of environmental toxicology. Available evidence indicates that interconversion of HCH isomers do occur in nature and can also be carried out in laboratory by employing conditions like high temperature, pressure and UV irradiation. Although much work has been done to study the transformation of HCH isomers in the environment and biota, the subject of interconversion has not received much attention. This paper reviews the work done on the interconversion of HCH isomers.

Introduction

The persistent organochlorine insecticide, hexachlorocyclohexane (HCH), popularly known as benzenehexachloride (BHC), has eight stereoisomers of which four, α-, β-, γ- and δ-, predominate in the technical products because of their having relatively strainless bonds. Although technical HCH has been in use world over for many years, studies on the transformation of HCH isomers have been confined mostly to the highly toxic γ-isomer while the remaining isomers, which constitute the major contents, have not received significant attention, probably so, because of their less toxic nature. Not many reports are available on the transformations of the three isomers α,- β- and δ- in the environment and biota. However, it appears from the available literature[1,2] that the transformation of these isomers would probably follow degradation pathways similar to those of the γ-isomer.

In recent years, several investigators have studied the transformations of HCH isomers and their metabolites in soils[3], plants[4], insects[5,6], mammals[7-11] and microbes[12-14]. As a result many new metabolites of HCH have been identified. A scheme for the transformation of γ-HCH based on the results of recent studies in various laboratories was recently proposed by Engst *et al*[2] (Fig. 1). A study of this metabolic scheme reveals that HCCH and HCCOL are the probable intermediates in the transformation of γ-HCH to TeCP and TCP respectively. Under conditions not favourable for oxidation, PCCH is formed which later degrades to various chlorobenzenes.

A review of work done on the transformation of HCH isomers at various laboratories indicates that their degradation produces two series of metabolites—the chlorobenzenes and chlorophenols, which finally degrade in soil to some chlorine, free partly volatile compounds.

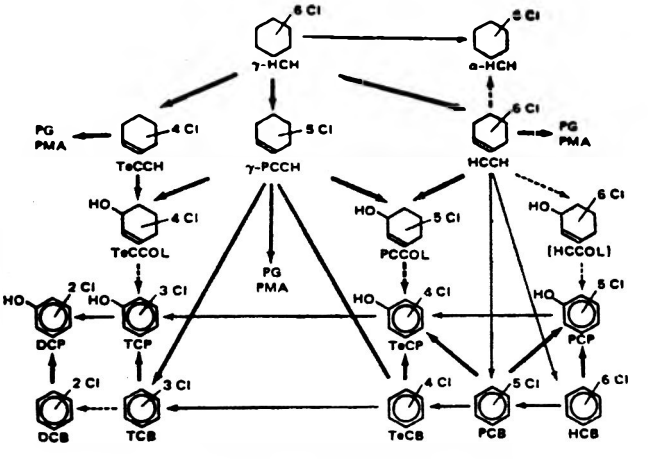

Fig. 1. HCB and gamma HCH including metabolic scheme by Engst *et al.*[2] (Reprinted with permission from *Die Nahrung;* copyright by Akademie—Verlag, Berlin, GDR).

Abbreviations used:

DCB	Dichlorobenzene
DCP	Dichlorophenol
HCB	Hexachlorobenzene
HCCH	Hexachlorocyclohexene
HCCOL	Hexachlorocyclohexenol
HCH	1,2,3,4,5,6—Hexachlorocyclohexane
PCB	Pentachlorobenzene
PCCH	2,3,4,5,6—Pentachlorocyclohexene
PCCOL	2,3,4,5,6—Pentachlorocyclohexene—(2)-ol-(1)
PCP	Pentachlorophenol
PG	Phenylglutathione
PMA	Phenylmercapturic acid
TCB	Trichlorobenzene
TCP	Trichlorophenol
TeCB	Tetrachlorobenzene
TeCCH	Tetrachlorocyclohexene
TeCCOL	Tetrachlorocyclohexenol
TeCP	Tetrachlorophenol

In few instances, the transformation of γ-HCH to HCB by microbes[15] and by plants[16] has been reported. A third possibility—the interconversion of HCH isomers, which may result in the gradual disappearance of a particular isomer and a simultaneous increase in the concentration of other isomers has not received much attention, although reports on the interconversion of HCH isomers in soils[17], plants[18], aquatic sediments[19], UV irradiation[20] and in aqueous solution[21,22] are available. This isomerization phenomenon may be quite important from the environmental toxicology standpoint, because there exists a significant difference between the HCH isomers in regard to their toxicity towards insects, pests, birds and mammals. The relative proportion of HCH isomers in the technical mixtures and their interconversion in the environment, therefore, needs attention.

Isomer composition during manufacture of technical HCH

HCH is prepared by chlorination of benzene in the presence of UV light[23]. The crude product thus obtained may consist of a mixture of eight chemically distinct isomers and one or more heptachlorocyclohexanes and octachlorocyclohexanes produced by the additive chlorination of mono and dichlorobenzenes during the reaction[24]. Out of the 16 theoretically possible isomers of HCH, the chair-chair interconversions reduce the number to eight only[25]. The analytical and structural determinations have validated the structure of the five isomers, namely ∝ –, β–, γ–, δ– and e–[26]. The eta and theta isomers were detected from the photochlorination of benzene tetrachloride[27]. Identification of the final member of the series, the iota isomer, initially referred as X_3-HCH, was claimed in 1969[28]. Further evidences, indicating that the new isomer was different from the earlier discovered seven isomers, in regard to its physical, chemical and biological properties were furnished in the subsequent publications[29–33]. The iota isomer is shown to have 3 chlorine atoms in the axil position and three in equatorial position[34]. The stereochemical configuration of HCH isomers is presented in Table 1. Of the isomers of HCH, only the γ-isomer is highly insecticidal. Hence, attention has been given to increasing the γ-content in the production of crude HCH. By utilizing different solvents, catalysts, high temperature, pressure and UV irradiation it has been possible to get the technical (HCH) product with varying isomer composition. Laugenbeck *et al*[35] have claimed that by using cyclohexyl peroxide and tetraisopentyltin, the chlorination reaction will proceed in darkness to give 25 per cent γ-isomer. Haen[36] has claimed a γ-isomer content of 30-50 per cent by cooling irradiated benzene. Low temperature chlorination at –8°C is said to result in odourless HCH[37]. An odourless and tasteless product has also been produced by refluxing technical HCH with $AlCl_3$ and extracting with petroleum ether[38].

Besides the chlorination in homogenous phase, benzene has been chlorinated in aqueous or alkaline dispersion. This process is difficult to control. However, the technical product has been claimed to contain upto 18 per cent γ-HCH. Gunther[39] used this method and prepared small batches of HCH by exhaustive chlorination of benzene emulsified in equal volume of 2 per cent aqueous NaOH. The mixture was irradiated for 75 hr with a powerful UV source giving wave lengths between 290 nm and the visible and the 295 g of semi-crystalline material produced contained 31.2, 0.9, 41.7 and 5.7 per cent of the ∝-, β–, γ– and δ–isomers respectively[39].

The addition at the chlorination stage of various compounds like carboxylic acids and their anhydrides, nitrotoluenes or the chlorides of sulphur, selenium or tellurium has been claimed to alter the isomer composition of technical HCH[40]. It is not clear whether the

TABLE 1. MOLECULAR CONFIGURATION AND PHYSIOLOGICAL EFFECTS OF THE ISOMERS OF HCH

Isomer	M.P.	New formulation (Trigonal carbon ring) a	b	Old formulation (Planar ring)	Physiological effects
Alfa	157	ppeeee	eepppp	↑↓↓↑↓↑ or 124	Weak excitant
Beta	297	eeeeee	pppppp	↑↓↑↓↑↓ or 135	Inert or weak depressant
Gamma	112	pppeee	eeeppp	↑↓↑↑↓↑ or 14	Strong excitant
Delta	130	peeeee	eppppp	↑↑↓↑↓↑ or 13	Strong depressant
Epsilon	219	peepee	eppepp	↑↑↓↓↓↑ or 123	Not insecticidal
Eta	90	ppepee	eepepp	↑↓↓↓↓↑ or 12	—
Theta	124	pepeee	epeppp	↑↑↑↑↓↑ or 1	Unknown
Iota	—	pepepe	epepep	↑↑↑↑↑↑ or 123456	Insecticidal

change in isomer composition is because of preferred chlorination of benzene to particular isomer or there is any secondary interconversion of the primary products formed. Nevertheless, these observations do suggest that interconversion of HCH isomers may possibly be achieved by employing certain reaction conditions.

Environmental Persistence

Many reports on the persistence and accumulation of β-HCH in soils and animal and plant tissues, following extensive use of technical HCH[41-43], are available. There are, however, some results which are contrary to these observations. Macrae and Raghu[44] while working with paddy soils in the Philippines observed that the persistence of all the four HCH isomers in submerged soils, under unsterile conditions, was essentially the same and no longer lasted beyond 50 days. Recently, Siddaramappa and Sethunathan[45] reported rapid degradation of γ-HCH and β-HCH in alluvial, laterite and pokkali soils under submerged puddled paddy soil conditions. In their work γ-HCH degraded faster than β-HCH. Recent report of the work done at Indian Agricultural Research Institute, New Delhi, India[46], on residues of HCH isomers in soils and crops indicates that there was no significant accumulation of any particular HCH isomer, either in soils or in crops, after extensive use of technical HCH, to cause any environmental problem.

Recently, in Japan, during studies on the disappearance of HCH isomers from water solutions in colourless wide mouth bottles exposed to sun light[47,48] the rates of disappearance of the isomers at 5 ppm concentration were in the order $\alpha \geq \beta > \gamma > \delta$. The half life of these isomers was:

α-HCH	.. ..	4–6 days
β-HCH	.. ..	4–9 days
γ-HCH	.. ..	5–11 days
δ-HCH	.. ..	10–22 days

In a recent survey of residues of organochlorine insecticides in some component of the Koso river, five years after the prohibition of their use[49], no accumulation of any particular HCH isomer was discovered. The Koso river water (per litre) contained 10-70 ng α-HCH, 5-40 ng γ-HCH, 10-30 ng β-HCH and 0-10 ng δ-HCH. These results suggest that there would be no significant accumulation of any HCH isomer, especially in aquatic environments. In recent years, Haider[50] while working on the transformation of HCH isomers by aerobic and anaerobic soil microorganisms reported that *Clostridium* and several representatives of *Bacillus* and *Enterobacteriaceae* effectively degraded HCH isomers to nearly chlorine free partly volatile metabolites. In his work γ-HCH was the most easily degraded isomer, while α-and especially β-and δ–were more slowly dechlorinated. These isomers were found as environmental conaminants, partly because of their persistence and partly due to the interconversion of γ–HCH to α-HCH and other isomers during microbial incubations.

Interconversions using drastic measures

The first report on the interconversion of HCH isomers was available through the excellent work of Wheatstone *et al.*[51] at the University of California. These workers observed interconversion of HCH isomers by heating them in a sealed tube with anhydrous $FeCl_3$ and other Friedel Craft catalysts under nitrogen at 140°C or above. In their work, the symmetrical β-isomer (135/246) was readily and exclusively converted to the α-isomer (124/356). The most stable was α-isomer which isomerized slowly and in low concentration to a mixture of isomers; γ-mostly isomerized to δ-with small quantities of α-, while δ-was found to give mostly α-and small quantities of γ-, although a small portion of δ–isomerized. Following the work of Wheatstone *et al.*[51], more reports on the interconversions of HCH isomers using drastic measures like high temperature, pressure and UV irradiation have appeared in recent years. Kawahara and Moku[52] reported that the four HCH isomers, α-, β-, γ- and δ-, in cigerette were interconvertible by heating. While working with pure HCH isomers, these workers observed different per centages of isomers in cigerette smoke and ash. In their work[52], α-HCH was converted to β–, γ– and δ–HCH and 1,2,4–TCB and 1,2,3–TCB on an evaporating dish under normal atmospheric pressure and at 260°C. Similarly, β–HCH isomerized to α-, γ– and δ–HCH in a quartz tube under pressure at 550°C. Recently, Roemer *et al.*[53]. have reported interconversion of α–HCH using high pressure. In their work, a solution of α-HCH in benzene containing 10-20 per cent $(Me_2N)_3$ PO at 10-12 K. bar and 180° for 8 hr gave 30 per cent of γ-HCH. Similarly, α-HCH in a 9:1 mixture of benzene and Me_2SO gave 90 per cent β-HCH. Steinwandter[20] observed isomerization of lindane to α-HCH by the action of UV light. In his experiment, a 2 hr irradiation of solid lindane and of lindane solutions in petroleum ether, acetone and water at 230 nm isomerized lindane to α-HCH by 2 per cent or less. A 5 hr irradiation in water induced complete disappearance of lindane, however, α-HCH was not found. Irradiation of solid lindane produced δ-HCH.

Interconversions in the environment

Soils: Japanese workers[42,43] have reported significant accumulation of β-HCH in soils, crops and in tissues of living organisms following extensive use of techni-

cal HCH which may contain 10 per cent β-HCH. Although reports indicating accumulation of β-HCH in soil and water are available, it is not clear whether all the β-HCH present in soils and crops resulted solely from the β-HCH present in the technical product or a part of it resulted from the isomerization of other HCH isomers. Irradiation with UV light of different wavelengths down to 230 nm shows that small amounts of α-HCH are formed as a major transformation product from other isomers.[54]

Aquatic environments: The phenomenon of interconversion of HCH isomers appears to be quite important in aquatic environment. Newland *et al*[19]. found both α-and δ-isomers in aquatic sediments incubated with pure γ-HCH. Similarly, Benezet and Matsumura[17] reported that microorganisms are capable of producing α-HCH from γ-HCH both in laboratory and in aquatic sediments. These workers isolated from soil a strain of *Pseudomonas putida* which converted γ-HCH to α-HCH in pure culture studies. This isomerization reaction was catalysed by NAD and to a lesser extent by FAD. According to these workers, it is most likely that the presence of high levels of α-and β-HCH in the environment may possibly be the result of isomerization of γ-HCH leaving behind residues of other constituents of HCH.

Plants: Steinwandter[55,56] recently observed interconversion of γ-HCH on grass and hay samples to HCB, α-HCH and β-HCH in laboratory studies. In another experiment with grass samples contaminated with γ-HCH, Steinwandter[57] observed that the formation of α-HCH was much faster than HCB in sun light, while in closed room the formation of HCB was relatively much faster. α-HCH subsequently changed, in part, to β-HCH. The formation of β-HCH was also much faster under field conditions than in closed room. Steinwandter, therefore, concluded that α-HCH, β-HCH and HCB could be found in the environment after γ-HCH application.

Karanth[58] incubated α-HCH with a crude enzyme preparation obtained from 10-day old tomato seedlings. TLC analysis of the mixture at the end of incubation time indicated presence of γ-HCH, β-HCH and PCCH.

Interconversion in aqueous solution

Recently Deo, *et al*[21,22] have observed that all the four HCH isomers, α-, β-, γ-and δ-, isomerize slowly and in small amounts, on dispersion in water at 25°C. This isomerization phenomenon was found to affect the toxicity of the aqueous solutions of individual isomers. Deo *et al*.[22] observed that the toxicity of aqueous solutions of α-, β-and δ-isomers gradually increased, while that of the γ-isomer decreased significantly with time during the 4-week experimental period. They also observed a significant loss of all the four isomers from their aqueous solutions at 25°C. These findings are very important from the stand point of environmental toxicology because of the significant changes in the toxicity of individual HCH isomers. Further, these observations support the view that HCH isomers may not perist much longer in aquatic environments.

Mechanism of interconversion

Many reports on the interconversion of HCH isomers are available. However, not many attempts have been made to explain the mechanism of isomerization. Wheatstone *et al*.[51] who observed interconversion of HCH isomers by heating them in a sealed tube under nitrogen at 140°C in the presence of $FeCl_3$, were the first to suggest a mechanism for the isomerization of HCH isomers. According to the suggested mechanism, taking β-HCH as an example, the catalyst $FeCl_3$ first abstracts one of the three chlorine atoms on, for illustration, the top side of the cyclohexane ring (Fig. 2.) The electron defecient carbon atom then forms a cyclic ion with either of the two adjacent carbon atoms and the attached chlorine atom. This chlorine atom and the cyclic ion are necessarily on the under side of the cyclohexane ring. Return of the chlorine atom to the original carbon atom reforms β-isomer, but return to any of the neighbouring

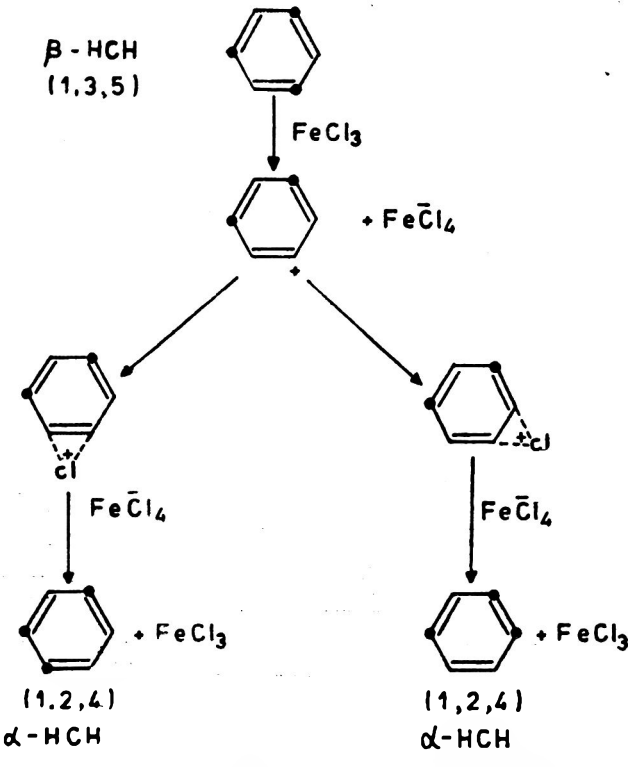

Fig. 2. Mechanism of interconversion of HCH isomers suggested by Wheatstone, *et al*.[51] A dot in the formula represents a chlorine atom above the plane of the ring.

participating carbon atom results in the ∝-isomer (124/356). Since all carbon atoms in the β-isomer (135/246) are equivalent, isomerization by this mechanism can give only the ∝ isomer as a primary product. With the other known isomers, in which all carbon atoms are not identical, formation of two or more isomers would be expected.

According to the mechanism suggested by Wheatstone *et al.*[51], a carbon atom can participate with each of its two neighbouring carbon atoms and thus four transformations are possible for each carbon atom or a total of 24 for all the six. Many of these transformations will, of course, reform the starting isomer. In this mechanism, the positions of both the abstracting and return of the chlorine atom would be influenced by stearic factors and selective formation of one or more isomers would be expected.

The products predicted from each of the four isomers by Wheatstone *et al.*[51] are shown in Table 2. The agreement of the observed results with those predicted was sufficiently good to support the proposed mechanism.

Newland *et al.*[19] explained the isomerization of γ-HCH to both ∝-and δ-isomers in aquatic sediments on the basis of the thermodynamic stabilities of the HCH isomers, chemical transformation of γ-isomer to more stable ∝-and δ-isomers.

Benezet and Matsumura[17] put forth the view that in their studies on the metabolism of γ-HCH with a strain of *Pseudomonas putida*, ∝-HCH was formed as a by-product of a complex NAD dependent pathway which involved production of large amounts of γ-BTC from γ-HCH.

Recently suggested mechanism by Engst *et al*[2] indicates HCCH as an intermediate in the conversion of lindane to ∝-HCH and HCB. The occurrence of HCB and HCCH as metabolites of lindane was partially noticed earlier[6,11]. The enzyme system catalysing this

TABLE 2. INTERCONVERSION OF HCH ISOMERS

Isomer	Predicted products[b]				
	∝	β	γ	δ	∈
∝	12	2	4	4	2
β	12	12	—	—	—
γ	8	8	—	8	—
δ	4	4	4	8	4(n-)

Wheatstone *et al.*[51]

b: Number of possible transformations out of the total of 24, which would give the indicated isomer.

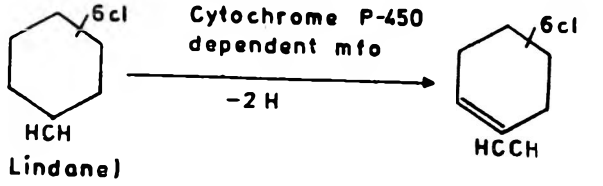

Fig. 3. Formation of HCCH from HCH

reaction was found to be localized in the microsomes of rat liver requiring molecular oxygen and a NADPH generating system. Therefore, the reaction is apparently a cytochrome-P-450 dependent mixed function oxidation. This oxenoid reagent generated from oxygen and a group of enzymes abstracts possibly two hydrogen atoms from the substrate (e.g. γ-isomers of HCH) to give the alkene HCCH and water directly (Fig. 3). A reversal of the reaction sequence in Fig. 3 will result in the reformation of HCH—but may form a different isomer.

Deo *et al.*[21,22] suggested that the interconversion of HCH isomers on dispersion in water was caused by the catalytic action of small quantities of HCl which resulted from the dehydrochlorination of HCH isomers by water at normal temperature. The action of HCl was considered by them similar to that of $FeCl_3$ in the work of Wheatstone *et al.*[51]

Conclusion

Currently available literature shows that interconversions of HCH isomers do occur in nature. The present knowledge on interconversion of HCH isomers is summarised in Fig. 4. It is possible that two types of mechanisms may exist—biological and non-biological. The non-biological interconversion may involve the

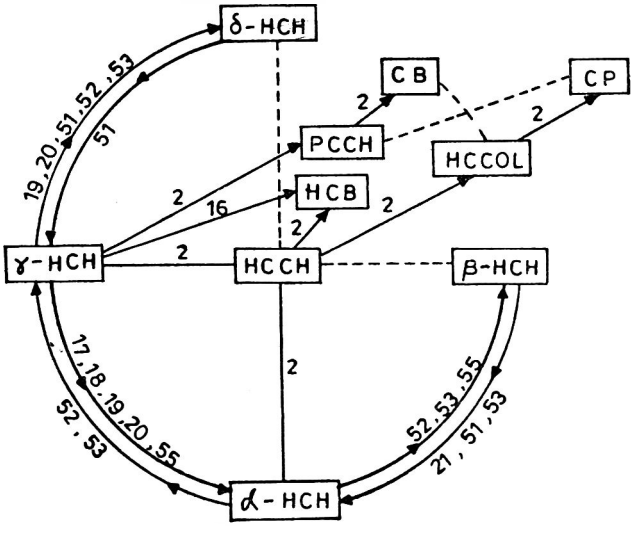

Fig. 4. Interconversion of HCH isomers—present position. Dotted lines indicate transformations which may possibly occur.

mechanism suggested by Wheatstone *et al.*[51], wherein a Friedel Craft catalyst like $FeCl_3$ or even HCl abstracts a chlorine from HCH and returns the same to the adjacent carbon atom thereby forming a new isomer. The biological interconversion may involve HCCH as an intermediate. The HCCH may either form a new isomer or may get further reduced to HCB. HCCH is also considered to be an intermediate in the transformation of HCH to chlorobenzenes and chlorophenols.

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BOOK REVIEWS

Advances in Biochemical Engineering, Bioenergy, Vol. 20: by A. Fiechter, Springer Verlag, Berlin, Heidelberg, New York, 1981; pp. 204; Price $ 38.20.

This publication is timed well with intensive developmental activities in various countries on bioenergy. This publication shows, besides the limitations, some realistic possibilities for the production of bioenergy from the biomass. In the wake of a 100 million dollar grant to the U.S. programme on "Fuels and biomass" and intensive R & D effort by several countries to produce ethanol from sugar and high polymer carbohydrates, as well as methane from agricultural waste and sewage sludge, this compilation of articles from several activiely working groups in different countries is most welcome. A wide range of topics such as (*i*) biochemical engineering for fuel production in the United States; (*ii*) structure and pretreatment and hydrolysis of cellulose; (*iii*) alcohol production and recovery; (*iv*) conversion of hemicellulose carbohydrates, and (*v*) production, economics and energy of the fuel ethanol from biomass have been covered. In addition, the status of bioconversion programmes in Brazil, Finland, West Germany, South Africa, Switzerland and Sweden have also been presented. This compilation is very useful to R & D workers in the area of lignocellulosic waste utilisation for bioenergy chemicals. Every topic has been dealt with in a masterly way and by its own merit can prove to be a very valuable guiding document. This also contains index for 1-20 volumes.

V. Sreenivasa Murthy,
C.F.T.R.I., Mysore.

Trends in Fish Utilisation: by J. J. Connel and R. Hardy, Fishing News Books Limited, Farnham, England, 1982; pp. 103; Price £ 6.

This is a Buckland Foundation Publication, one of a series providing a record of annual lectures. The book deals with the more effective utilisation of fish and shell fish. The authors who are working in Torry Research Station have given a comprehensive description of the different marine animals as source of food which were not considered useful before. When marine fishing started, fishes were caught indiscriminately making people choosy. This resulted in some species being rejected and only parts of others being utilised. The authors have pinpointed the unused and little used marine animal resources which the U.K. can utilise. The emphasis is on the direct human consumption rather than for animal feeding. By suitable technical manipulations, edible material can be obtained from offal which is now dumped at sea or used as fish meal. According to the authors, the total potential catch within the EEC's economic zone of fish and shell fish whose flesh is likely to be basically acceptable to consumers plus the recoverable marine animal protein add up to at least twice that which are used at present. And the source is renewable.

The book has 5 chapters. The first chapter gives a history of the fishing and fishing industry developments. Second chapter gives details about the unused and underused resources. Details of some sixteen species of fish and shell fishes are given. In this chapter, the authors have presented the existing trends in fish consumption in U.K. and methods are suggested to improve the industry. The ways in which U K might go about manipulating and modifying these resources are discussed in chapters 3 and 4. Greater utilisation of fish catch can be achieved by improving or developing conventional methods which involves avoidable spoilage, deterioration and waste. Because of the steeply rising price of fish, the processing industry has to take steps to use as much as possible of what they procure. The last chapter deals with price competition, marketing patterns, consumer attitudes, business promotion and export potential.

Although the report surveys the situation in U.K., it can be of use to other countries also. The printing and get up of the book are good. For those interested in further reading, a bibliography is included at the end of the book.

T. R. Sharma,
Defence Food Research Laboratory, Mysore

The Quality of foods and beverages. Vol. 2 *Chemistry and Technology:* Ed. by: George Charalambous and George Inglett, Academic Press, 1982: pp. 390;

This is the second volume of the proceedings of the Second International Flavour Conference held in July 1981 in Athens (Greece). The book is a collection of articles of widely varying interests from the use of computer for pre-evaluation of model systems in the reaction of furfural with H_2S and NH_3 to cell-meditaed immune responses of carotenoids, and include aspects of chemistry, raw materials, technology, commercial development of flavours, analysis and quality control. The topics include composition of smoke and preparation

of smoke flavours; enzymatic flavour developments in foods; enhancing fruit flavours in desserts using many volatile and non-volatile organic compounds, inorganic salts and products of thermal degradation of sugar; furans and pyrazine derivatives as important components of roasted high grown Arabica coffee; presence of elements like As, Br, Cl, Cu, Mn, K and Na in commercial red and white Greek wines, their permissible levels and direct or indirect influence on the quality.

Information on developments in some countries relate to changes in diet patterns of Japanese with westernization, increasing use of wheat flour foods and expectation on wheat quality; use of dried grapes, prunes and figs in California to prepare concentrates and pastes and their uses; state of enology and viticulture in California; need in Sweeden for traditional, well tasting prepared convenience foods of good nutritional value consequent on 75 per cent of the women going to work and more than 50 per cent of the population living in urban areas.

Aspects of technological development covered are: novel food proteins from legumes and oil seeds currently available in Italy to extend meat; flavouring of extrusion cooked and textured meat extenders from defatted soy flour; advances made in the processing of legumes to prepare convenience, fortified products, enriched bakery products and milk substitutes; advances in soy sauce manufacture and the product being free from any carcinogenic constituents; quality evaluation of macadamia nut used as cocktail nut and in confectionery trade on account of unique texture and flavour; stability and odour and flavour of spices and their oleoresins in stored food products; moisture relations to the growth of microorganisms; and interaction of lipid constituents in flours, concentrates and isolates from oil seeds with water.

Articles relating to analysis and quality control are: translating laboratory analytical procedures to on-line methods; integrators and computers for a modern flavour laboratory; mechanized plating instrument for use in the determination of viable counts of microorganisms in foods and other biological materials; gas chromatographic method for the determination of cocoa butter substitutes in chocolate; application of HPLC for evaluation of coffee flavour quality; and pollution of liquid food by PVC containers.

Approach for development of flavours is well illustrated in the paper by Manfred H. Vock of International Flavours and Fragrances. The book contains a number of figures, useful tables and subject index. The get up is good, but the arrangement of topics could have been better. It is useful as a reference book for research workers in the field to widen their knowledge.

S. RANGANNA,
C.F.T.R.I., MYSORE.

Food Process Engineering: by Dennis R. Heldman and R. Paul Singh, AVI Publishing Company Inc., Westport, 1981 Connecticut, 2nd Ed., pp. 415, Price $ 26.50.

The contents of this book have been brought out under various unit operations, which are normally found in the undergraduate books of chemical engineering.

After an introductory chapter, the book deals with theology of food products, heating and cooling processes, thermodynamics of food freezing, evaporation for fluid food concentration, food dehydration, contact equilibrium and mechanical separation processes.

There is a long felt need for the design of food process equipment and machinery in the changed context of the energy crisis and consequent conservation of energy in food processing industry. In all the chapters, the authors have given importance to the design aspects of equipment and machinery, wherein the data from the batch processes could easily be applied to the continuous processes. The authors have also attempted to use the SI units and this may be very much helpful for the teachers and students.

The foremost job of food technologist/engineer is to supply wholesome nutritious food to the consumer by judicious usage of suitable technology and equipment specially in the third world countries. The authors have attempted to deal with this aspect in greater detail. Since most of the unit operations developed and standardised by chemical engineering schools used the concepts for the standardisation of equipment based on the design data, when intensive energy sources were abundantly available, this book will help to stimulate the thinking of food engineers to look into various design parameters primarily for the reduction of process and product cost.

The presentation is extremely good and the book will be very much useful as a ready reference for research workers in the field of food processing industries and undergraduate students of food engineering.

M. M. KRISHNAIAH,
C.F.T.R.I., MYSORE.

Proceedings of the Workshop on the Management of R & D Institutions in the Area of Food Science & Technology: Sponsored by the United Nations University held at the Central Food Technological Research Institute, Mysore on July 17-24, 1979, published by the United Nations University Programmes, Central Food Technological Research Institute, Mysore, 1979, pp. 220.

Management of R & D institutions is becoming increasingly complex and professionalised. It has proved

the fact that a successful and eminent scientist/academician need not necessarily meet with the same degree of success at the helm of directing research and managing a multidisciplinary and result oriented institution. The need for achieving well defined goals in the shortest possible period while working in a highly systemised time schedule and accountability have further emphasised the necessity of a professional manager with a clear vision and perspective.

A workshop on the management of R & D institutions was held at Central Food Technological Research Institute, Mysore, India to provide a forum to share the experience of different participants and to gain an insight into the measurement of R & D institutions. Twenty eight participants and 4 observers from 16 countries or international agencies who are actually in charge of cost, budgeting, communicating, management and futurology participated. This book, under review, is a compilation of the papers, presented and the discussion that followed.

The ten chapters include (1) theme and goal of the workshop, (2) pre-and post harvest operations; (3) requirements of R & D institutions; (4) structure of R & D institutions; (5) factors influencing R & D activity (6) project selection and future problem areas; (7) technology evaluation and transfer; (8) communication and information; (9) financial matters; and (10) staff problems: A panel discussion.

Mr. C. P. Natarajan has opened the workshop with a paper on the experience of CFTRI in the management of Food Science and Technology. The paper outlines the growth, present structure, working and future outlook of this premier institution.

Chapter 2 on pre and post harvest operations contains two papers by F. G. Winarno of Indonesia and S. K. Majumder of India. Both the authors have presented a bird's eye view of the problem connected with pre and post harvest operations in their respective countries.

The specific requirements, expectations and objectives of the R & D institutions situated in Mexico, Thailand, Bangladesh and Tocklai Tea Research Station, India are covered in the third chapter.

The structure and organisation of the National Food Research Institute of Japan, Food Research Institute, Ghana and the Meat Research Institute, Denmark are given in the chapter 4.

The hard pressed problem of the management of a research institution is to choose basic and applied research. The next chapter focusses on the difficulties of this choice. The importance of basic research and the interdependence of basic and applied research are brought out in two papers. To avoid technological obsolescence and with the ultimate aim of common meal to society, basic research has to be accommodated, within financial and scientific manpower constraints. These constrains are discussed in the subsequent papers. Political factors generally influence the national R & D activity, especially in the case of laboratories attached to government which is a feature in most of the developing countries. Illustration of such factors changing the scientific priority, the criteria for problem identification and the influencing factors have been well presented by Dr. G. S. Sidhu of R.R.L. Hyderabad. These have been further brought home with appropriate examples with specific reference to project selection and future areas in the two subsequent papers of the succeeding chapter.

Any technological development has to pass through the critical phase of technology evaluation and transfer. Evaluating R & D institutions is also a complex task. Dr. K. T. Achaya has presented a paper on the above subject, taking C.F.T.R.I. as a case study. A similar experience of Nigeria was projected by O.A. Koleoso of Federal Institute of Industrial Research.

The problems and quantification of the technology transfer are dealt with by C.V.S. Ratnam of ESCAP Regional Centre for Technology Transfer, Bangalore, India and Dr. B. L. Amla of the World Bank.

Communication and information is an intellectual bank. Proper dissemination is of paramount importance. Development of information system and knowledge management as an integral input of R & D and the library and documentation services in India are covered in this chapter.

Financial management and staff problem are comprehensively discussed in the last two chapters.

This book is a valuable compilation of the views and experiences of the experts from various countries. It should be indispensable for those who have to face day-to-day management problems of R & D institution in general and food science and technology in particular.

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ASSOCIATION NEWS

Annual General Body Meeting

The 17th Annual General Body Meeting of the Association of Food Scientists and Technologists (India) was held on 17th June 1982 at CFTRI, Mysore, India. Mr. M. K. Panduranga Setty, President of the Association was in the chair. Dr. L.V. Venkataraman, Hony. Exec. Secretary presented the report of the activities of the Association during 1981–82. During the year the membership increased from 1488 to 1890. Four new chapters were started at Poona, Karnal, Nagpur and Palayamkottai (Tamilnadu). Presently there are 15 chapters of the Association.

The International Food Conference (Ahara 82) and Exhibition (Expo) held at Bangalore during last week of May 1982 was the major event of the Association. During the Conference which had a focal theme on the Food Challenges of Eighties was attended by 1380 delegates including over 50 delegates from 20 foreign countries. The Conference was inaugurated by the President of India, Mr. Neelam Sanjeeva Reddy and a keynote address on the 'Global Food Situation' was delivered by Dr. M. S. Swaminathan, Director General of International Rice Research Centre at Philippines and former member of Planning Commission. Six symposia were held on the following topics: (1) Innovative methods of food storage and preservation, (2) Food quality and standards, (3) Natural and man-made toxicants, (4) Fermented foods, (5) Training needs and facilities and (6) Food packaging. Three panel discussions on the following topics were held: (1) Placing of food technology in meeting the needs of developing countries in achieving self-sufficney, (2) Constraints in developing food industries in the third world and (3) Conveyance of nutrition to the low-income groups and experience with mass produced nutritive food. Besides, 350 research papers were presented as posters. All the past and present Presidents, Secretaries and Editors of the Association were honoured as part of the Silver Jubilee Year.

A New journal of the Association 'Indian Food Industry' Vol. 1 Nos. 1 & 2 was released during the Ahara 82. This journal is specially meant for food industries, its management and R & D personnel connected with food industries in India. The journal is issued quarterly and contains two review articles in different areas of food industry and technology, food industry developments, notes and news.

The Expo 82 organised at Bangalore palace grounds had the participation from government and nongovernment agencies, commercial and noncommercial sectors, consumer oriented products, convenience foods, export oriented products, food machinery and equipment. Nearly 60,000 people visited the weeklong food exhibition.

The Secretary informed the members that the first presentation of Dr. H. A. B. Parpia award will be made during the Silver Jubilee Year (1982–83). The Secretary also presented the activities of the various chapters of the Association. He pointed out that efforts must be made by the various chapter office bearers to collect subscriptions promptly from members.

The Hony. Treasurer presented his report for the year as also the budget estimate for the year 1982–83.

The Secretary's report and Treasurer's report were unanimously approved by the General Body.

As part of the AGBM, award of AFST(I) for 1982 were distributed by the President of the Association Mr. M. K. Panduranga Setty.

(*i*) Prof. V. Subrahmanyan Industrial Achievement award: *Recipient*—Dr. T.N. Ramachandra Rao

(*ii*) Gardners award (for the best Scientific paper published in JFST—1980).
Recipients—Drs. C. S. Narayanan, M. A. Sumathikutty, K. Rajaraman, B. Sankarikutty, A. V. Bhat and A. G. Mathew.

(*iii*) Best Student award—
Recipients—Mr. Prakash Raghunath Bhirud and Mr. K. M. Nagargoja

(*iv*) Suman Food Consultants Travel award—
Recipient—Ms. H. R. Vijaya

(*v*) Young Scientist award—Not awarded.

Dr. T. N Ramachandra Rao delivered a lecture entitled "Microbial Bio-technology—Problems, Progress, and Promise".

The Office bearers of the Association for the year 1982–83 were announced by the Hony. Exec. Secretary.

President	Mr. S. K. Majumder
President-elect	Lt. Col. O. P. Kapur
Vice-President—HQ	To be nominated later
Vice-Presidents	Dr. A. S. Aiyar
	Dr. G. A. Sulebele
	Dr. C. S. Naik Kurade
	Dr. M. S. Laul
Hony.Exec.Secretary	Dr. L. V. Venkataraman
Hony.Jt.Secretary	Dr. S. C. Basappa
Hony.Treasurer	Mr. S. V. Ramakrishna
Hony.Editor (JFST)	Dr. R. Radhakrishna Murty

Dr. L. V. Venkataraman proposed the vote of thanks and congratulated the outgoing President Mr. M. K.

Panduranga Setty for his efforts in organising the Ahara 82 and Expo. He also thanked the executive members and AFST(I) members for the successful completion of activities for the year 1981–82.

New executives of AFST(I) for 1982–83

The Central Executive Committee of the Association met on 26th July 1982 at CFTRI, Mysore and nominated the following executives to fill up the existing vacancies—

1. *Vice-President (Head quarters)*

   Dr. T. R Sharma, Director, Defence Food Research Laboratory, Mysore.

2. *Hony.Treasurer*

   Mr. C. T. Dwarakanath, Scientist, CFTRI, Mysore will succeed Mr. S. V. Ramakrishna who has resigned consequent to his getting appointment in RRL, Trivandrum.

3. *Hony.Editor (Journal of Food Science and Technology)*

   Dr. K. R. Srikantiah will assume office of Editor of the Journal of Food Science and Technology from January 1983 for a period of three years. He will succeed Dr. R. Radhakrishna Murty, whose term ends by December 1982.

Ludhiana Chapter

The following were elected as the Office bearers for 1982 in the Annual General Body Meeting of the Chapter held on July 22, 1982, President—Dr. M. S. Kalra, Vice-President—Dr. K. L. Gaba, Secretary—Dr. K. S. Sekhon, Joint Secretary—Sri B. S. Ahluwalia and Treasurer—Dr. H. P. S. Nagi.

The Chapter plans to hold a seminar on 'Baking Industry' in November 1982 and an essay writing competition among the student members.

Nominations Invited for

AFST(I) Awards for 1982

Prof. V. Subrahmanyan Industrial Achievement Award

The Award consists of a cash prize of Rs. 2500, a citation and a plaque.

The guidelines for the award are as follows:

1. Indian nationals engaged in the field of Food Science and Technology will be considered for the award.
2. The nominee should have contributed to the field of Food Science and Technology, for the development of agro-based food science and technology with immediate prospect and (or future potential) for industrial application.
3. The nomination should be proposed by a member of the Association. The biodata of the candidate together with his consent should be given in detail including the work done by him and for which he is to be considered for the award.

Young Scientist Award

The award consists of a cash prize of Rs. 1000, a plaque and a citation.

Nomination for the Award is open to aspirants fulfilling the following conditions:

1. The candidate should be an Indian national below the age of 35 years on the date of application, working in the area of food science and technology.
2. 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 candidates own contribution to the work: OR
   (*b*) Technological contributions of a high order, for example in product development, process design etc., substantiated with documentary evidence.

Best Student Award

There are two awards each comprising a cash award of Rs. 500 and a certificate.

The candidates to be considered for the awards should fulfill the following conditions:

1. They must be Indian nationals.
2. They must be students of one of the following:
   (*a*) M.Sc. (Food Science)/Food Technology
   (*b*) B.Tech., B.Sc. Tech., B.Sc. Chem. Tech in Food Technology
   (c) B.Tech., in food sciences
3. They should not have completed 25 years of age on 31st December of the year preceding the announcement when their names are sponsored.

Heads of Post-graduate Departments in Food Science and Technology 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 his postgraduate performance to date.

Nominations or applications for the awards along with bio-data and contributions should be sent by registered post covers marked "awards" so as to reach Dr. L. V. VENKATARAMAN, Honorary Executive Secretary, Association of Food Scientists and Technologists (India), Central Food Technological Research Institute campus, Mysore-570 013 before 31 january 1983.

Suman Food Consultants Travel Award AFST(I)—1981-82

The Association of Food Scientists and Technologists (India) have instituted a Travel Award in the name of "Suman Food Consultants" to Post-graduate Degree/Diploma students in Food Science/Technology. The Award will be of Rs. 500 (Rupees five hundred only) which will enable the awardee to attend the Annual General Body Meeting and the Technical Seminar/Symposium of the AFST (I) in that year.

The selection for the Award will be based on an essay competition. The subject will be announced later.

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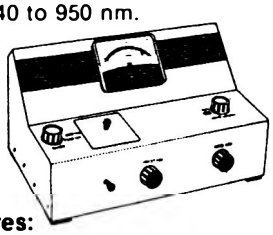

Features:
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- High sensitive phototubes (Blue and Red) as detectors premounted with selector knob

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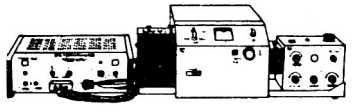

Features:
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Atomic Absorption Spectrophotometer AAS4103

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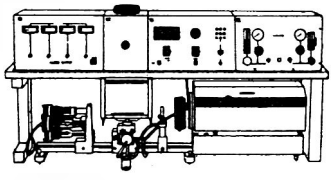

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- Digital readout of absorbance, transmittance, concentration

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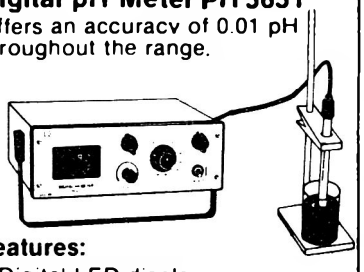

Features:
- Digital LED display
- High degree of accuracy
- Range 0 to 14 pH and 0 to ± 1999 mv
- Readout — Digital LED display

Fluorometer FL6209

Fluorescence from 1 ppm quinine sulphate in 0.1N sulphuric acid (primary 366 nm) produces full scale voltage

Features:
- Mercury lamp as radiation source
- High sensitive photomultiplier detector
- 3½ digit digital display

Mercury Analyser MA5800A

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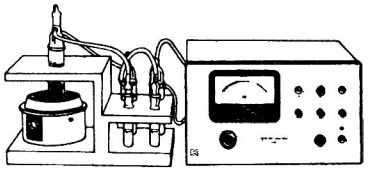

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INSTRUCTIONS TO AUTHORS

1. Manuscripts of papers (*in triplicate*) should be typewritten in double space on one side of bond paper. The manuscripts should be complete and in final form, since only minor corrections are allowed at the proof stage. The paper submitted should not have been published or data communicated for publication anywhere else. Review papers are also accepted.

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. 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. It should be 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 paper. 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. The lettering should be in double the size of printed letters. For satisfactory reproduction, graphs and line drawings should be at least twice the printed size 16cms (ox axis) × 20cms (oy axis); 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 Calcicolous 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 copy of the *Journal* for guidance.

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Research Papers

Research Notes

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