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ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS

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AFST(I) will be conducting the First AFST International Food Conference 1982 in Bangalore from Sunday May 23 to Wednesday May 26, 1982.

The inauguration will coincide with the Silver Jubilee Function of AFST(I) and will be held on the evening of Sunday May 23, followed by a dinner. A plenary lecture will be featured at the start of each day, and two popular evening lectures are also scheduled. Outstanding speakers will be invited to give these talks. There will be a total of 6 symposia and 3 panel discussions. These will take up the rest of the mornings.

Research papers will be presented in poster sessions, which will be spread over the afternoons of all the three days. Any topic in the area of food science and technology in its broadest sense can form the subject of these poster papers. Thus, apart from basic and applied fields covering food commodities and processing, such aspects as teaching and training, nutrition, toxicology, packaging, environment and by-products, standards and regulations, information science, technology transfer, marketing and economics, are also included.

An exhibition of food machinery and food products will be organised along with the Conference. A book fair emphasising technical books and literature will also be featured.

The theme of the Conference will be "Food challenges of the eighties". A special attempt is being made to seek participation from developing countries, and the symposia and panel discussions will be slanted towards their problems. The choice of topics for the plenary and popular lectures will be left to the expert speakers themselves, to ensure authoritative treatment.

For details regarding all aspects of the conference, please write to: M.R. Chandrasekhara Convener, Planning Group, First AFST(I) International Food Conference 1982, 19, Platform Road, Bangalore-560 023, India (Telegrams: SNACKFOODS, Telephone: 82430).

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RESEARCH PAPERS

A Simple Key for the Identification of *Bacillus* Species Common in Foods

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An artificial key based on biochemical tests is presented to aid in the identification of *Bacillus* species commonly encountered in foods. The key is specially designed for rapid confirmation of *B. cereus* isolated on selective media. The species are divided into two groups based on their ability to utilize the citrate salts.

The genus *Bacillus* is a physiologically heterogenous assemblage with at least 48 species described in the Bergey's Manual.[1] It is relatively simple and straight forward to recognize the genus as comprising of aerobic sporeforming Gram positive rods that are also mostly catalase positive. Characterization of the species belonging to this genus involves lengthy procedures and several biochemical tests. In view of facilitating a rapid identification, the species are often treated in two or more groups[1-3]. This is mainly based on the position and shape of the spore and swelling of the sporangium. This treatment, however, does not limit the biochemical tests. Consequently in reports pertaining to survey of bacteria in different foods, it is not uncommon to find the isolates being only identified as "*Bacillus* spp". In an attempt to curtail the number of biochemical tests, and to expedite the identification, the following key is presented. Of the different species of *Bacillus*, reportedly *B. cereus* is becoming increasingly important in suspected foods involved in food poisoning[4,5]. Hence in this key priority is mainly towards a rapid identification of this species.

The Key

Citrate utilization:
+.....Group A.
—.....Group B.

Group A
Acid from arabinose:
—.....*B. cereus*
+.....Refer A-1.

A-1:
Starch hydrolysis:
—.....*B. pumilus*
+.....Refer A-2.

A-2:
Anaerobic growth:
+.....*B. licheniformis*
—.....Refer A-3.

A-3.
Acetoin production:
+.....*B. subtilis*
—.....*B. megaterium*

Group B
Gelatin liquefaction:
+.....Refer B-1.
—.....Refer B-2.

B-1:
Acetoin production:
+.....Refer B-la.
—.....Refer B-lb.

B-la:
Nitrate reduction:
+.....*B. polymyxa.*
—.....*B. alvei*

B-lb:
Starch hydrolysis:
—.....Refer B-lbi.
+.....Refer B-lbii.

B-lbi:
Nitrate reduction:
+.....*B. laterosporus*
—.....*B. badius*

B-Ibii:
Acid from xylose:
+.....*B. macerans*
—.....Refer B-Ic.
B-Ic:
Phenylalanine deamination:
+.....*B. firmus*
—.....Refer B-Ici.
B-Ici:
Acid from mannitol:
+.....*B. circulans*
—.....*B. stearothermophilous*
B-2:
Starch hydrolysis:
—.....*B. sphaericus*
+.....Refer B-2a.
B-2a:
Urease test:
+.....*B. lentus*
—.....*B. coagulans*

Using this key and performing biochemical tests as outlined in the Bergey's Manual[1] 150 isolates of *Bacillus* from different species were identified[6]. Simultaneously several standard cultures of *Bacillus* maintained in the biology department at the University of Waterloo were also tested. The biochemical responses of the isolates were in agreement with the reactions listed in manuals[1,7] and a report by Knight and Proom[8]. The key was further reevaluated at the senior author's present address using *B. cereus* NCTC 11143, *B. cereus* NCTC 11145, *B. licheniformis* NCTC 10341, *B. coagulans* NCTC 10334, and *B. subtilis* NCTC 5398, obtained from the Central Public Health Laboratory, London.

The division of *Bacillus* in this key into Group-A and Group-B is totally artificial and is only for convenience. Importance is given to *B. cereus*. As treated in this key, *B. cereus*, simply stated, is a citrate-positive *Bacillus* that cannot ferment arabinose. For isolating *B.cereus* there are several selective media such as the egg yolk polymyxin medium (KG medium)[9], and the MYP medium[10]. Identification using the above media although tentative, is based on the production of lecithinase by the organism. The formation of a zone of precipitation around the colony on the egg yolk agar plates constitutes positive reaction for *B. cereus*. But the occurrence of *B. cereus* strains that produce very narrow zones of precipitation and occurrence of lecithinase negative ones[11] renders the selective media inconclusive. It is suggested that such isolates be subjected to biochemical tests as proposed in the key.

Since the entire classification in the key is based mainly on the utilization of citrate salts, it is to be expected that the citrate variable species or strains would naturally pose problems. But, these species are very few, and include *B. anthracis*, *B. pantothenticus*, *B. brevis* and few strains of *B. coagulans*. Of these *B. anthracis* is found in meat from infected animals, and it is suggested that while screening meat for this organism, the scheme presented in the Bergey's Manual[1] may be used. *B. pantothenticus* although mainly distributed in soil[1], can occur as a contaminant in food. The Bergey's Manual should be used in identifying this organism. *B. brevis* although may be expected in food, it is problematic as far as the biochemical responses are concerned. In addition to citrate variability, acid formation from carbohydrates is a less dependable test since, reportedly[1], the reactions may be positive, weak or not reported for several strains. It is, therefore, suggested that when an isolate is suspected to be *B. brevis*, it may simultaneously be compared with reactions of type strains *B. brevis* ATCC 8246 or NCTC 2611. *B. coagulans* is included in Group-B in the key, as most of the strains are citrate-negative. It may, however, be noted that three per cent of the strains are positive for this test[7].

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Extracellular Production of Folic Acid Factor by the Mushroom *Coprinus lagopus*

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A yellow coloured mushroom, *Coprinus lagopus* when grown under submerged condition in synthetic medium produces yellow pigment in the broth. It was found to be a mixture of two compounds both having 6-amino pteridine as core moiety. The broth was found to have no folate activity but inhibits growth of various bacteria, maximally that of folate dependent *S. faecalis* and *L. casei*. Growth inhibitory activity may be antagonised by addition of excess folic acid in the medium. Production of yellow pigment in broth ceases and consequently antifolate activity of the broth disappears when fermentation was carried out in dark in presence of reducing agent (ascorbic acid). Under this condition high folic acid production (24.646 μ g/g dry wt.) was observed.

Folates have been isolated from cells of various microorganisms (algae, yeast, bacteria) and also from the mammalian liver[1,2]. But little information is available on the extracellular production of folic acid during fermentation. The present paper reports on the fermentative production of folic acid during submerged propagation of a yellow coloured mushroom, *Coprinus lagopus* (Fr) in the synthetic medium[3].

Materials and Methods

Chemicals: Folic acid (pteroylglutamic acid) was the product of Sigma Chemical Company, U.S.A., other chemicals used were of either AR or GR quality.

(a) *Mushroom strain and growth condition:* Mushroom used is a strain of *Coprinus lagopus* (Fr) which has already been reported[3] to grow well under submerged condition in shake flask in the synthetic medium of following composition (g/100 ml): starch (soluble), 6; urea, 0.032; KH_2PO_4, 0.087; boric acid, 0.057; $MgSO_4$, $7H_2O$, 0.05; $CaCl_2$, $2H_2O$, 0.037; $MnCl_2$, $4H_2O$, 0,0036; $NaMoO_4$; $4H_2O$, 0.0033; $ZnSO_4$, $7H_2O$, 0.03; $FeSO_4$, $7H_2O$, 0.025 at pH 5.0 and in temperatures between 29 and 31°C. During growth in shake flasks, it produces bright yellow pigment in the broth.

(b) *Paper chromatography of the fermented broth:* After 10 days of fermentation the broth was concentrated by lyophilization and applied directly on Whatman (3MM) paper and chromatographed in dark chamber in the following ascending solvent systems[4], (*i*) n-propanol: 1% ammonia solution (2: 1, v/v), and (*ii*) 3% (w/v) ammonium chloride solution.

(c) *UV-spectrum*: The UV spectrum analyses were made at different pH values using 0.1 N HCl (pH 1.0), 0.05 M phosphate buffer (pH 7.0) and 0.1 N NaOH (pH 13.0) in a Zeiss (Speckord) double beam spectrophotometer.

(d) *Alkaline permanganate hydrolysis*: Alkaline hydrolysis of folic acid and fermented broth were performed according to standard procedure[5] and the products were analysed chromatographically in the same solvent systems as stated earlier.

(e) *Assay of folic acid activity*: Both extracellular and intracellular folic acid activity was assayed[6,7] using *Lactobacillus casei* ATCC 7469 and *Streptococcus faecalis* ATCC 8043 as test organisms. In the assay condition, no conjugase treatment for breaking of polyglutamyl chain of folic acid was carried out[8].

(*f*) *Assay of growth inhibitory activity of the broth*: Growth inhibitory activity was measured turbidimetrically at 660 nm. Culture filtrate was sterilized by millipore filtration. *L. casei* and *S. faecalis* were grown in specific folic acid assay medium[6] with 0.1 ng/ml (*L. casei*) and 0.9 ng/ml (*S. faecalis*) of folic acid and other organisms in the nutrient broth for 12 hr. One drop of washed cell suspension was used as inoculum per 10 ml of assay broth containing different amounts of culture filtrate.

Fermentative conditions: Fermentation was carried out in the same medium for 10 days under two different conditions: one set in dark flask (100 ml/500 ml flask) with the addition of 2 mg/ml of ascorbic acid in the medium and another set without any such precautions.

Results and Discussion

The yellow coloured mushroom, *Coprinus lagopus* (Fr.) grows well in shake flask in synthetic medium[3] and produces yellow pigment in the fermented broth. Attempt at the identification of the colour compound(s) reveals the presence of two distinct coloured spots having UV-fluorescence. These two spots (designated as compound I and II, has Rf values of 10 and 20 in solvent system (a) and 58 and 50 in solvent system (b) respectively) when purified from the concentrated fermented broth by paper chromatography, showed following UV-absorbtion maxima at various pH values: compound I, λmax (pH 1) 272 and 372, λmax (pH 7) 265, λmax (pH 13) 260; compound II, λmax (pH 1) 242, 290 and 360, λmax (pH 7) 282, λmax (pH 13) 260 and 285. This observation indicates the presence of pteridine ring in the compounds I and II. With these findings it appears reasonable to analyse the alkaline permanganate oxidation product of the compounds to identify whether both the compounds have a common naturally occurring 6-substituted pteridine as their core moiety. With folic acid as reference compound, the UV-absorbing spots obtained after alkaline hydrolysis, either of compound I or II or of folic acid were found to be identical. Thus it appeared that although both the compounds I and II, differed from folic acid, are related to the vitamin and may have the biological activity. But it was found that the broth has no folic acid activity. In addition, it appears very surprising that the growth of the folic acid requiring strains, in presence of sufficient exogenous folic acid required for their growth are being inhibited by the presence of test broth in their growth medium. Growth inhibitory activity of the broth was also assayed against a Gram-positive and some of the Gram-negative organisms which do not require exogenous folic acid for growth (Table 1). It was observed that although some retardation of growth was noticed for these strains with relatively higher percentage of broth added in the assay system the inhibitory action is negligible compared to those observed for *L. casei* and *S. faecalis*. It was further noted that addition of higher amounts of folic acid i.e., greater than 0.1 ng/ml against 0.1% broth for *L. casei* and 0.9 ng/ml against 0.5% broth for *S. faecalis* support the growth of both the dependent organisms.

Now considering the light and oxidation sensitivity of folic acid, there appeared other probabilities, that extracellular folic acid might have been converted into some antifolate compounds or its synthesis was being inhibited at earlier stages under the influence of light and oxidizing conditions during fermentation.

To overcome this, precautions were undertaken during fermentation to minimise any conversion of folic acid, if produced, by light or by aeration. It was then observed that the fermented broth has no growth inhibitory activity against any of the organisms tested earlier and there is a marked decrease in the production of yellow pigment. On the other hand, the broth added in the assay medium, excellentely supports growth of folic acid requiring *S. facealis* and *L. casei*. The extracellular[6] and intracellular[7] folic acid activity was then measured (Table 2). Now within the limitation of available microbiological methods for the assay of total folate derivative in solution, the total extra-and intracellular folic acid content of 100 ml fermented broth (myceliae yield, 1 g dry wt.) was found to be relatively high (24.646 μg/g dry wt.) compared to the highest activity (20 μg/g of dry wt.) reported for brewer's yeast.[9]

TABLE 1. GROWTH INHIBITORY ACTIVITY OF FERMENTED BROTH

Organism used	* % of fermented broth supplemented in assay medium	% growth inhibited compared to normal (av)
Vibrio cholerae NG 214	5	77
Vibrio cholerae NG 6	5	50
Escherichia coli K 12	5	56
Shigella dysenteriae II	5	30
Staphylococcus aureus 8530	5	45
Salmonella uganda 9101	10	30
Shigella sonnei 56	10	56
Streptococcus faecalis ATCC 8043	0.5	100
Lactobacillus casei ATCC 7469	0.1	100

*Percentages added to obtain appreciable growth inhibitions.
Data represented are the averages of three observations.

TABLE 2. ASSAY OF EXTRA- AND INTRACELLULAR FOLIC ACID FACTOR IN FERMENTATION BROTH

Test organism	Folate activity[1]		Total activity (μg/100 ml fermentation)
	Intracellular (μg/g)	Extracellular broth (μg/100 ml)	
S. faecalis ATCC 8043	2.054	5.00	7.054
L. casei ATCC 7469	6.846	17.80	24.646

1. After inoculation in a 500 ml Erlenmeyer flask containing 100 ml of liquid media, an average of one gram (dry wt) of mushroom mycelia is obtained after 10 days of growth period. Data are the average of three replications.

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The Relation between Growth Pattern and Endogenous Metabolites in the Developing Fruit of Early Maturing Flordasun Peach

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The first and the second rapid growth stages lasted five weeks each while the middle slow growth lasted for two weeks. The total acidity had reached its peak by the end of stage II which subsequently declined. The amount of total phenolics in the flesh remained high during stage I and declined during stage II, and attained the maximum concentration with the inception of stage III. The total anthocyanins increased as the fruit ripening advanced. The flesh had more starch and total water soluble carbohydrates than the rest of the fruit. Out of this, glucose was the main component followed by sucrose and fructose. The level of total sugars remained high during stage II. The ascorbic acid content was maximum during stage I and it declined to a minimum level during stage II and increased considerably during stage III.

New peach cultivars have been introduced from the U. S. A. and their cultivation is on the increase. It is desirable that an insight into the developmental physiology of this fruit is obtained especially due to its very sensitive, quick ripening and perishable nature so that efforts can be made to facilitate its handling.

Some studies on the fruit growth of peach[1,2], on the qualitative and quantitative changes of sugars[3], titratable acidity[4], phenolic compounds[5], starch and ascorbic acid[6] have recently been reported in the development phases or at the onset of maturity upto its ripening. However, little published work on the endogenous level of metabolites in 'Flordasun' cultivar is available in India. The present study was, therefore, initiated to gain some insight into the changes of different endogenous metabolities beginning from anthesis to the ripeness stage.

Materials and Methods

Samples of the 'Flordasun' peach fruits were taken at weekly intervals commencing from one week after full bloom to ripeness. To determine the growth pattern of peach fruit, the length and diameter of ten fruits which were still on the tree was taken. The total acidity was

estimated by titrating the extract with 0.1 N NaOH using phenolphthalein as the indicator. The total phenolics and total anthocyanins were estimated by the methods as described by Swain and Hillis[7], and Pirie and Mullins[8] During 1977, the starch was estimated from the fruit by the method of Yemm and Willis[9], while during 1978,the same was estimated from the flesh by the colorimetric iodine method. To estimate the total water soluble carbohydrates, the fruit (stony and fleshy pericarp) during 1977 and flesh (during 1978) were extracted in 80 per cent ethanol by heating over a water bath until no sugar could be detected in the residue with anthrone reagent. The extract was clarified using saturated lead acetate solution[10]. The excess of the lead acetate was removed by adding sodium oxalate powder. The clear solution was used for colorimetric determination of carbohydrates using the anthrone reagent method of Yemm and Willis[9]. The sugars were estimated by the method as described by Shallenberger and Moores[11]. Ascorbic acid was estimated by the usual method[10].

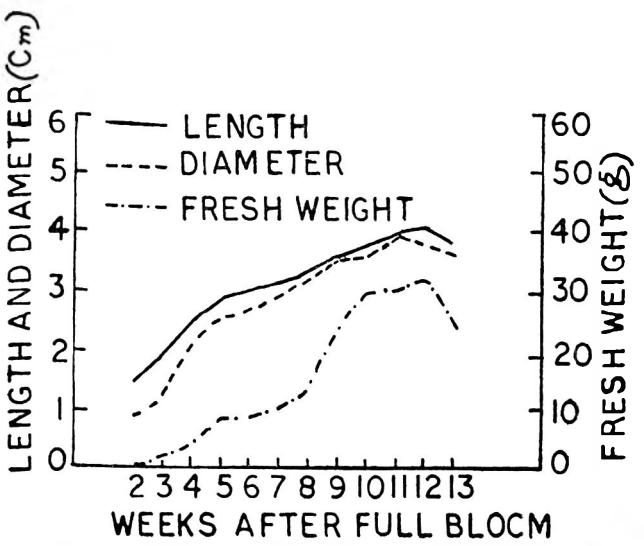

Fig. 1A. Growth pattern in 'Flordasun' peach fruit during 1977.

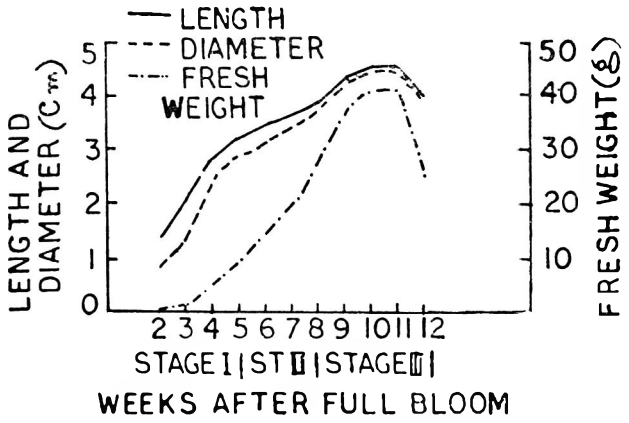

Fig. 1B. Growth pattern in 'Flordasun' peach fruit during 1978.

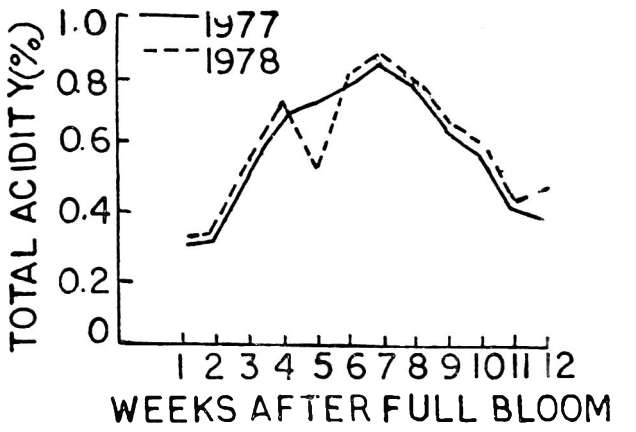

Fig. 1C. Time course changes in the total acidity in the flesh of 'Flordasun' during 1977 and 1978.

Results and Discussion

Growth pattern: There were two phases of active growth which were separated by a period of little or even no growth[1,2,12]. During 1977 and 1978, the growth pattern of 'Flordasun' fruit followed a double sigmoid growth curve (Fig. 1A, 1B). It has been further stressed that in peach, the difference in the fruit growth between the early and the late maturing cultivars was mainly in the relative length of middle slow growth stages[13,14]. In the early cultivars, this stage was almost absent where the embryo had aborted. In the late cultivars, the stage II was very prominent and the embryo had fully developed[15]. Further, the duration of the second stage was not the same in each cultivar and in turn determined the time of its ripening and the final fruit weight[12]. The length and diameter of peach fruits observed in 1977 and 1978 supported the view of Bollard[13], Ninkovski[12], Tukey[14] and Tukey[15] to the extent that in 'Flordasun' the slow growth phase during 1977 and 1978 was barely discernible and lasted only for two weeks. Thus, on account of this discernible lag phase, 'Flordasun' fruit ripened earlier in the season. The increase in fresh weight in 'Flordasun' also followed a double sigmoid curve, but the measurement of length and/or diameter was a better parameter to express the growth pattern in this cultivar.

Total acidity: It is evident from Fig. 1C that the level of organic acids in the flesh increased through stage I and II and reached its peak towards the end of stage II. There was rapid fall in the organic acids as the fruit ripened which were reduced to 0.39 and 0.48 per cent during 1977 and 1978 respectively. Similarly, the organic acids levels in peaches and nectarines reached

their maximum at maturity[1,4,6] and then decreased as the fruit ripened. The high level of organic acids in the developing fruits may be due to the low temperature prevailing at that time, since organic acids are formed at low temperature and are metabolized in respiration at high temperature at the time of ripening[16].

Total phenolics: The amount of total phenolics in the flesh (Fig. 1D) remained high in the beginning of stage I (maximum peak during 1977) and then continued to decline up to the end of stage II. It then rose sharply and readily attained its maximum at the inception of stage III in 1978. Presumably, the presence of low levels of monophenolics during stage II which could serve as a weak co-factor for IAA-oxidation resulting in the increased amount of auxins[17] and thus shortened the slow growth phase. The total phenolics were considerably reduced at maturity indicating their hydrolysis into other components[6].

Total anthocyanins: The flesh contained higher total anthocyanins at the beginning (Fig. 2 A) which was probably due to the inclusion of some flower parts. After a depression during the first stage, it had markedly increased through stage II and stage III attaining the peak at the end of stage III. The anthocyanin pigments in peach were reported to markedly increase as the fruit ripened on the tree[18,19].

Starch: The starch content in the 'Flordasun' fruit during 1977 (Fig. 2B) decreased continuously till the middle of stage I and then it fluctuated through the rest of the period, which indicated that either the starch was not accumulated or its accumulation was followed by its immediate hydrolysis. During 1978, the level of starch in the 'Flordasun' flesh fluctuated during stage I, but it remained steady during stage II and most of the stage III period. The highest starch content was obtained at the end of stage III which finally declined due to its hydrolysis into sugars[6]. Probably on account of high starch content in the flesh especially during the stage II, the slow growth phase was shorter in 'Flordasun' fruit.

The flesh had higher amount of starch than rest of the fruit throughout the fruit development. The qualitative changes in the concentration of starch throughout the growing season indicated its rapid increase during the first few weeks of growth, reaching its maximum thereafter and completely disappeared later in the season when fruit matured[1,20]. Similarly , observation was made in the present investigation, that the starch in fruit or flesh did not disappear completely.

Total water soluble carbohydrates: There was almost a continuous increase in the level of total water soluble carbohydrates in fruit throughout its development period during 1977 except some casual decline during stage II and III (Fig. 2C). Similar observation was made in 1978 also. The flesh contained greater amount of total water soluble carbohydrates than in fruit throughout development period. There appeared to be a direct correlation between starch and total water soluble carbohydrates in the fruit and flesh, except during stage II in fruit, when these metabolites showed inverse relationship during this phase.

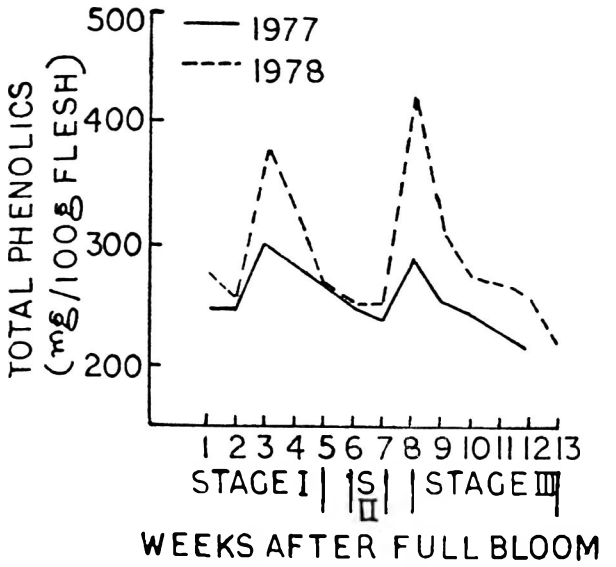

Fig. 1D. Time course changes in the total phenolics in the flesh of 'Flordasun' during 1977 and 1978.

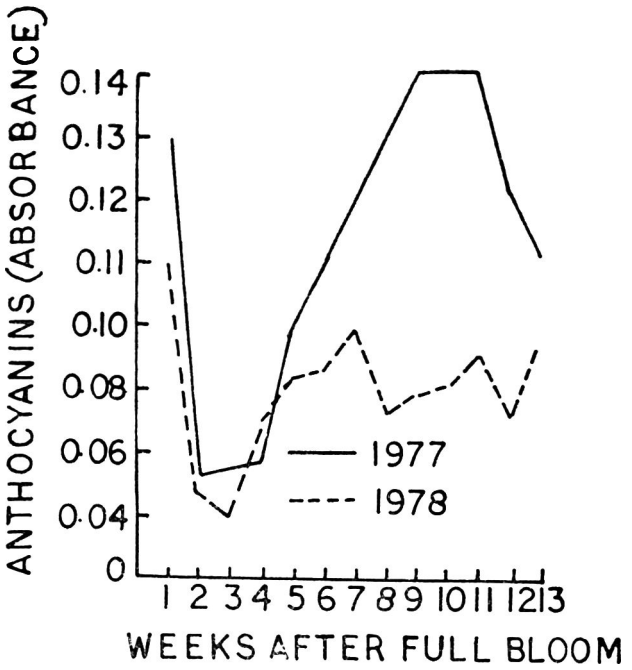

Fig. 2A. Time course changes in the total anthocyanins in the flesh of 'Flordasun' during 1977 and 1978.

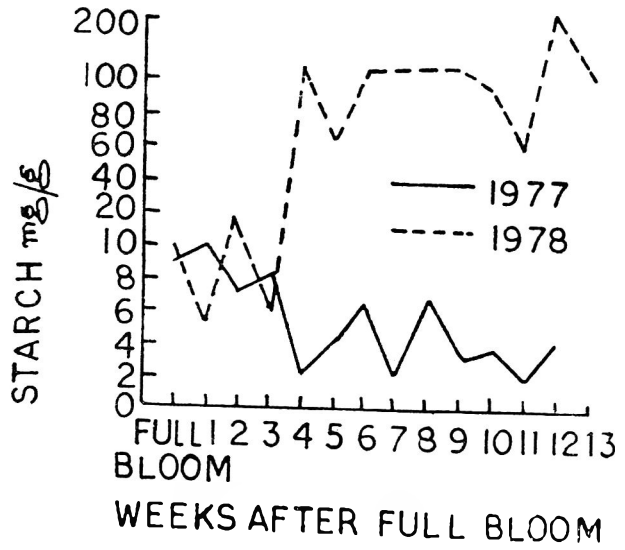

Fig. 2B. Time course changes in the starch in the entire fruit (1977) and flesh (1978) of 'Flordasun' fruit.

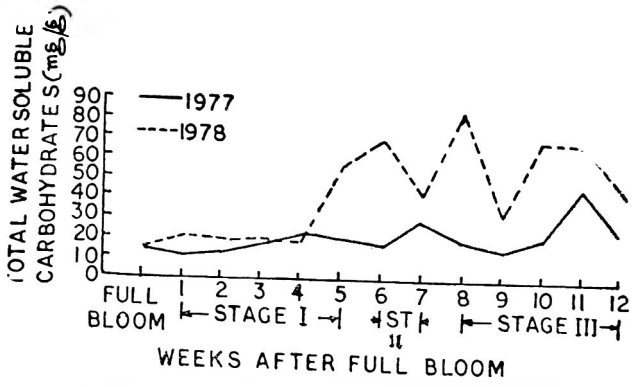

Fig. 2C. Time course changes in the total water soluble carbohydrates in the entire fruit (1977) and flesh (1978) of 'Flordasun' fruit.

TABLE 1. CHANGES IN THE LEVEL OF SUGARS IN THE DEVELOPING FRUIT AND FLESH OF 'FLORDASUN'

Fruit Developmental Stages	Duration after full bloom (weeks)	Sucrose (mg/g)	Glucose (mg/g)	Fructose (mg/g)	Total sugars (mg/g)
		Fruit 1977			
Stage I	1–5	36.10	4.85	—	40.95
Stage II	6–7	15.95	6.75	—	22.70
Stage III	8–13	47.80	38.58	13.38	99.75
		Flesh 1978			
State I	1–5	20.80	51.17	10.00	81.97
Stage II	6–7	7.70	43.10	12.95	63.65
Stage III	8–13	97.40	254.50	23.00	374.90

Sugars: Sucrose was the main sugar in the fruit of 'Flordasun' (Table 1) and its concentration exceeded that of total reducing sugars[21]. With the exception of sucrose during stage II, the sucrose and glucose increased continuously in the fruit through all the stages of development.[22,6] Fructose appeared only during stage III. The level of total sugars remained high during stage I, declined during stage II and it was the maximum during stage III.

Glucose was the main sugar (Table 1) followed by sucrose and fructose in the flesh of 'flordasun' during 1978. The level of sucrose, glucose and total sugars was high during stage I, which declined during stage II and attained its peak during stage III. However, fructose increased continuously through all stages of development.

During stages I and II, sucrose was high in the fruit but flesh had higher sucrose during stage III. Greater amount of glucose, fructose and total sugars was produced in the flesh than in the fruit during all stages of fruit development.

There was an inverse relationship between starch and glucose during stages I and II while it altered at stage III in both the fruit and the flesh.

During stage III, there existed an inverse relationship between total acidity, and between total phenolics on the one hand and total sugars on the other, in both flesh and fruit, indicating that orgnic acids and total phenolics were hydrolyzed into sugars[6].

Hence, it may be suggested that on account of greater amount of sugars, available to the developing flesh during stage II, the lag phase was less discernible and lasted only for two weeks.

Ascorbic acid: During 1977 (Table 2), the ascorbic acid continuously decreased in the flesh throughout fruit development. However, during 1978, its content remained consistently high during stage I and III but declined to low level during stage II.

It is quite clear that the ascorbic acid content was significantly influenced by the season and the stage of maturity.

The earlier findings that the rise in the ascorbic acid towards maturation did not seem to be a general feature in peach fruit[6,16] support the present investi-

TABLE 2. CHANGES IN THE ASCORBIC ACID CONTENT IN THE FLESH OF 'FLORDASUN'

Fruit development stages	Duration after full (weeks)	Ascorbic acid(mg/100 g flesh)	
		1977	1978
State I	1–5	41.5	22.8
Stage II	6–7	4.9	6.5
Stage III	8–13	10.5	22.4

gation. However, Dhuria *et al.*[4], Salunkhe *et al.*[23] and Zubeckis[24] reported an increase in the ascorbic acid content with the increasing maturation of the fruits on the trees which was also observed in the present investigation during 1978.

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Effect of Water Activity on Storage Changes in Total Carotenoids and Lipids in Bengalgram *(Cicer arietinum)* Dhal and Flour

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Bengalgram flour and dhal were equilibrated to 2.0, 8.0, 10.8 and 13.9% moisture at 0.0, 0.33, 0.57 and 0.73 water activity (a_w) respectively. Storage below 0.57 a_w does not cause perceptible changes in flavour for 24-52 weeks but on storage at 0.73 a_w became moldy and developed musty odour in 8 weeks storage. Below 0.57 a_w, changes in total carotenoids, TBA value, free and bound lipids and their composition were not significant except slight hydrolysis of triglycerides and a concomitant increase in fat acidity. Both free and bound lipids and carotenoids were degraded during storage at 0.73 a_w. Proportions of neutral, glyco and phospholipids in Bengalgram flour are 93, 3 and 4% in free lipids and 15.8, 16.9 and 66.9% in bound lipids respectively. Proportion of phospholipids in bound lipids decreased while neutral lipids increased during storage at 0.73 a_w due to mold infection.

Bengalgram is quite resistant to chemical spoilage but storage of Bengalgram flour results in musty, rancid and bitter flavour. In cereals and legumes, lipids are most susceptible to storage deterioration and their degradation has been correlated with off flavour development. Kowsalya and Kantharaj Urs[1] have reported that storage of raw, roasted and puffed Bengalgram flour is associated with both autoxidative and hydrolytic degradation of lipids. Since both these changes are influenced by water activity (a_w) in the product[2], its influence on the changes in total carotenoids, free and bound lipids and their composition during

storage of Bengalgram flour and dhal were investigated.

Materials and Methods

Bengalgram dhal and flour: Bengalgram, three months after harvest, was dehusked and split. This was ground in a mill to pass through 60 mesh to get the flour.

Storage: One hundred and fifty grams were stored in petri dishes (15 cm diameter) in desiccators having anhydrous potassium pentoxide and saturated solutions of magnesium chloride, potassium bromide and sodium nitrate to obtain water activities of 0.00, 0.33, 0.57 and 0.73 respectively. All the desiccators were kept at room temperature (20-35°C) away from direct sunlight.

Analysis: Initially and periodically, the samples were analysed for moisture, fat acidity, thiobarbituric acid (TBA), free and bound lipids and their composition. Moisture content and fat acidity were determined according to standard AACC procedures[3]. TBA value was determined by steam distillation procedure of Tarledgis *et al*[4]. For determining total carotenoids, 2 g samples were treated with 50 ml acetone and kept in dark for one hour and filtered under suction. The residue was washed three times with 10 ml portions of acetone. The combined extract was treated with 30 ml hexane and freed from acetone by washing with aqueous sodium chloride solution (5%). The hexane layer was made to 50 ml and treated with a pinch of anhydrous sodium sulphate to remove moisture. The concentration of total carotenoids was calculated from the absorbance at 449 nm using $E^{1\%}_{449}$ 2500.

Free lipids from the samples were extracted with petroleum ether (40-60°C) in a soxhlet extractor for 16 hr. The extract was evaporated to dryness under vacuum, freed from moisture over anhydrous sodium sulphate and made up to 50 ml with chloroform. Lipid concentration was determined gravimetrically by evaporating 5 ml portions of the solution to constant weight at 100°C.

For determining bound lipids, 20 g petroleum ether extracted samples were shaken with 200 ml water saturated n-butanol for 3 hr and filtered under suction. The residue was washed successively with 100 ml and 50 ml of n-butanol. The combined extract was evaporated to dryness and dissolved in 120 ml chloroform-methanol (2:1) mixture. The non lipid impurities were removed by Folch wash procedure[5]. Extract was freed from moisture by treating with anhydrous sodium sulphate and concentration of lipids was determined gravimetrically by drying 5 ml aliquots of the lipid solution.

Both free and bound lipids were further fractionated into neutral lipids, glycolipids and phospholipids by silicic acid column chromatography according to the method of Rouser *et al*.[5] Recovery in column chromatographic separations varied from 99.5 to 101.9%. Thin layer chromatography was performed on silica gel G plates activated at 120°C for half an hour using chloroform-methanol-water (65:25:4) and petroleum ether-diethyl ether-acetic acid (85:15:1) as solvents for the separation of polar and non polar components. Individual lipid components were characterised by co-chromatography with authentic compounds and by specific spray reagents for glycolipids and phospholipids[6].

Results and Discussion

Bengalgram flour and dhal on storage at 0.0, 0.33, 0.57 and 0.73 a_w had an equilibrium moisture content of 2.0 ± 0.1, 8.0 ± 0.1, 10.8 ± 0.2 and 13.9 ± 0.2 per cent respectively. During storage of flour for 24 weeks and dhal for 52 weeks, no musty or rancid flavours were perceptible in samples stored at 0.57 a_w or below. But in samples stored at 0.73 a_w, visible mould growth appeared within 8 weeks of storage with concomitant disappearance of yellow colour and development of musty odour.

Changes in total carotenoids during storage of Bengalgram flour and dhal are shown in Table I. Freshly milled Bengalgram flour had about 30 μg/g total caro-

TABLE 1. EFFECT OF WATER ACTIVITY ON TOTAL CAROTENOIDS, TBA VALUE AND FAT ACIDITY OF BENGALGRAM FLOUR DURING STORAGE AT ROOM TEMPERATURE (20-35°C).

Water activity	12 weeks storage			24 weeks storage			52 weeks storage		
	Carotenoids (μg/g)	TBA value	Fat acidity (mgKOH/100g)	Carotenoids (μg/g)	TBA Value	Fat acidity (mgKOH/100g)	Carotenoids (μg/g)	TBA value	Fat acidity (mgKOH/100g)
0.00	30.0	40.6	19.9	29.0 (30.0)	40.5	23.8(21.3)	(28.0)	—	(21.0)
0.33	29.8	36.5	32.1	28.1 (29.8)	36.9	37.0(26.8)	(26.0)	—	(30.8)
0.57	28.9	40.9	61.1	28.0 (29.5)	38.3	76.5(37.0)	(26.0)	—	(48.5)
0.73	12.8	64.9	184.9	10.4 (26.0)	248.1	25.9(69.8)	(20.0)	—	(105.9)

Initial values: Carotenoids (μg/g flour), 30.2; TBA value (μg/malonaldehyde/g lipid), 35.3; Fat acidity (mg potassium hydroxide/100g flour), 20.8. Values in parenthesis are for stored dhal.

tenoids which decreased very slightly during storage below 0.57 a_w. At a water activity of 0.73, total carotenoid level decreased to a considerable extent and the rate of decrease was faster in flour than in dhal. Increased rate of carotenoid destruction at higher water activities has been reported by Quackenbush[7], Bailey *et al*[8], and Arya and Parihar[9]. In isolated systems, however, the rate of carotenoid destruction has been found to decrease with increasing water activities[10]. Higher rate of carotenoid destruction at elevated moisture levels may therefore, be attributed to the native enzymes or to molds.

There were only slight changes in TBA value of Bengalgram flour and dhal having water activity less than 0.57. But the samples stored at 0.73 a_w showed a significant increase in TBA value during storage. Evidently despite high proportions of polyunsaturated fatty acids in Bengalgram dhal, atmospheric autoxidation of lipids is not appreciable; major deteriorative changes being brought about by native enzymes or by growing molds. This is also supported by changes in fat acidity and free and bound lipids (Tables 1 and 2). It may be observed that fat acidity did not change to a significant extent in samples stored at 0.0 a_w whereas in the samples stored at a_w of 0.33 and 0.57 it increased slightly. In the samples stored at 0.73 a_w, Bengalgram lipids were extensively hydrolysed resulting in a very rapid increase in fat acidity. However, beyond 12 weeks' of storage at 0.73 a_w, fat acidity began to decline with a concomitant decrease in both free and bound lipids. After 24 weeks' of storage about 90% of free lipids and and 33% of bound lipids had disappeared. Obviously, this is due to molds, as has been observed in mold damaged wheat flour previously[9,11,12]. Relatively, the changes in fat acidity, free and bound lipids were considerably smaller in dhal than in flour.

Both free and bound lipids were further separated into neutral lipids, glycolipids and phospholipids on silicic acid column. Bengalgram free lipids comprise mostly neutral lipids (93%); phospholipids (4.0%) and glycolipids (3%) being present in relatively small proportions. On the other hand phospholipids (66.9%) are the major constituents of bound lipids; the neutral and glycolipids being 15.8 and 16.9% respectively. Storage at 0.57 a_w and below did not result in significant changes in the proportions of neutral lipids, glycolipids and phospholipids in the bound lipids but at 0.73 a_w, the proportion of phospholipids decreased while that of neutral lipids and glycolipids increased significantly both in dhal and flour (Table 3). The apparent increase in glycolipids may be due to epoxy and hydroxy fatty acids, presumably formed during storage, which are known to elute with glycolipids due to their increased polarity.

Further separation by thin layer chromatography revealed that neutral fraction of free lipids comprise mostly triglycerides, diglycerides, monoglycerides with free fatty acids being present only as minor constituents. On the other hand free fatty acids are present in greater proportions in the neutral fraction of bound lipids both in fresh and stored samples, while triglycerides, diglycerides and monoglycerides could be detected only in trace quantities. There were no significant changes in the composition of bound neutral lipids but proportion of triglycerides decreased appreciably in free neutral lipids on storage.This change was especially very pronounced in samples stored at 0.73 a_w.

Major polar components of free and bound lipids were phosphatidyl serine, phosphatidyl inositol, phosphatidyl choline, lysophosphatidyl choline and monogalactosyl diglyceride. Phosphatidyl ethanolamine and digalactosyl diglyceride were present in bound lipids but absent in free lipids. Proportions of major polar components did not change significantly in samples stored below a a_w of 0.57 but in samples stored at 0.73 a_w, polar lipids were degraded considerably.

TABLE 2. EFFECT OF WATER ACTIVITY ON FREE AND BOUND LIPIDS OF BENGALGRAM FLOUR AT ROOM TEMPERATURE (20–35°C).

Water	12 Wk storage		24 Wk storage		52 Wk storage	
	Free lipids (%)	Bound lipids (%)	Free lipids (%)	Bound lipids (%)	Free lipids (%)	Bound lipids (%)
0.00	5.25	1.93	5.27	1.92	5.13	2.05
0.33	5.28	1.99	5.29	1.94	5.11	2.03
0.57	5.32	1.96	5.34	1.86	5.16	2.01
0.73	4.11	2.06	0.54	1.27	4.84	1.69

*Free and bound lipids in dhal. Initial values: free lipids, 5.20%; bound lipids, 2.00%.

TABLE 3. EFFECT OF WATER ACTIVITY ON THE COMPOSITION OF BOUND LIPIDS DURING STORAGE OF BENGALGRAM FLOUR AND DHAL

Water activity	12 wk stored flour			52 wk stored Dhal		
	Neutral (%)	Glyco (%)	Phospho (%)	Neutral (%)	Clyco (%)	Phospho (%)
0.00	15.8	17.2	65.5	16.4	18.0	66.3
0.33	15.9	18.7	65.7	16.7	18.4	65.9
0.57	16.3	19.7	65.9	15.9	19.4	65.7
0.73	24.1	36.6	39.8	27.2	34.8	38.6

Initial values: neutral lipids 15.8%; glycolipids 16.9%; phospholipids 66.9%.

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Developmental Changes in Kernel Fractions and Dietary Fibre of Normal and Opaque-2 Maize *(Zea mays L.)*

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Marked differences in crude fibre, neutral detergent fibre, acid detergent fibre and hemicellulose content were observed in normal ('Ganga-5') and Opaque-2 maize whole kernel, as well as in their pericarp and endosperm. In both normal and Opaque-2 pericarp, various fibre fractions increased, but in whole kernel, only crude fibre decreased with kernel development. In normal kernel, maximum quantity of neutral detergent fibre and hemicellulose were recorded after 30 days of pollination stage, whereas in Opaque-2 they were observed 35 days after pollination. The level of acid detergent fibre was maximum at 35 days both in Opaque-2 and normal kernels. In both normal and Opaque-2, per cent pericarp decreased whereas per cent embryo increased during development. The Opaque-2 kernel contained lesser pericarp and higher proportion of embryo than normal maize kernels.

Currently there is a growing awareness of the nutritional significance of indigestible components of the diet, mainly of unavailable carbohydrates made up of crude fibre (cellulose and lignin[1]), hemicellulose (precursor of cellulose[2]) and pectic substances. This group of substances is often referred to as "dietary fibre". These are not readily digested by secretion of the human digestive system[1-3]. Dietary fibre of plant origin, specially of cereals, is receiving considerable attention as an essential nutrient that has beneficial effect on hypercholesterolimia and various other disorders[4,5].

In an earlier study[6], involving 'Ganga-5' and 'Shakti' varieties, the protein content as well as biological value (BV) was observed to decrease from 25 days after pollination to kernel maturity. On the other hand true digestibility (TD) of protein improved with increase in age of kernel development in both varieties. The pericarp fraction which is primarily composed of relatively indigestible component, decreased with the age of kernel development. This study was undertaken to determine if the observed relationship between true digestibility and biological value was in any way related to dietary fibre content.

Materials and Methods

'Shakti Opaque-2' composite and a normal high yielding hybrid 'Ganga-5', varieties were used in the study. These two varieties were grown at the Indian Agricultural Research Institute, New Delhi, in the monsoon season of 1979 and each plant was self-pollinated, and develo-

ping ears of each variety were harvested at 25, 30 and 35 days after pollination and also at maturity. Each sample at each stage of development consisted of 5-10 well filled ears. The endosperm, embryo and pericarp were separated from about 100 g kernel at each stage of development. The kernel fractions, as well as whole kernel of all stages, were dried at 50°C until 10 per cent moisture level was reached.

Crude fibre (CF) was determined according to the AACC[7] method. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analyzed by the method of Goering and Van Soest[8]. Hemicellulose (HC) was determined, as suggested by Van Soest and Moore[9], by difference between NDF and ADF fraction. Indigestible content (IDC) was determined[10]. The dried fat-extracted sample were ground to pass through a 1-mm screen. A sample of 1g was used for CF, 0.5g for NDF and another 0.5g for ADF determinations.

Results

Developmental changes in kernel fraction: The per cent pericarp of the kernel decreased (Table 1) whereas embryo per cent increased during development in both normal and 'Opaque-2' varieties. The embryo fraction increased from 12.59 to 16.45 per cent while the pericarp fraction decreased from 10.05 to 7.00 per cent from 25 days after pollination to maturity in normal maize, while in 'Opaque-2' it was 8.69 to 9.73 and 12. 48 to 9.30 per cent respectively. The ratio of pericarp to embryo was almost twice in normal compared to Opaque-2 at all stages of kernel development. The pericarp weight in case of Opaque-2 was 7-8 mg per kernel lower than that of normal in all stages of kernel development; Opaque-2 had larger embryo fraction and its size increased with development. The endosperm, the major fraction, did not show appreciable changes during development in both normal and Opaque-2, but its proportion was found to be slightly higher in normal than opaque-2 at all the stages of development. The weight of endosperm per kernel, however, increased gradually during development in both normal and Opaque-2.

TABLE 1. CHANGES IN THE PROPORTION OF KERNEL FRACTION DURING DEVELOPMENT IN NORMAL AND OPAQUE-2 MAIZE.

Maize Var.	Days after pollination	Endosperm (%)	Embryo (E) (%)	Pericarp (P) (%)	P/E
Shakti (opaque-2)	25	77.4 (116.0)	12.6 (18.9)	10.0 (15.1)	0.80
	30	77.0 (131.6)	14.3 (24.3)	8.7 (14.9)	0.61
	35	76.3 (137.3)	16.6 (29.8)	7.6 (13.7)	0.46
	Mature	76.6 (150.0)	16.5 (32.2)	7.0 (13.7)	0.43
Ganga-5 (Normal)	25	78.8 (146.4)	8.7 (16.1)	12.5 (23.2)	1.44
	30	80.1 (174.6)	9.7 (21.1)	10.2 (22.3)	1.06
	35	80.9 (181.3)	9.4 (21.1)	9.6 (21.5)	1.02
	Mature	81.0 (181.5)	9.7 (21.8)	9.3 (20.9)	0.96

Figures in the parentheses indicate wt. in mg/kernel.

TABLE 2. DIETARY FIBRE IN DEVELOPING NORMAL AND OPAQUE-2 MAIZE PERICARP

Type of fibre	Shakti (Opaque-2)				Ganga-5 (normal)			
	25 days	30 days	35 days	Maturity	25 days	30 days	35 days	Maturity
Crude fibre (%)	11.90 ± 0.006 (1.80)	12.08 ± 0.012 (1.80)	12.93 ± 0.002 (1.80(	13.50 ± 0.006 (1.85)	13.30 ± 0.012 (3.09)	15.10 ± 0.058 (3.37)	16.04 ± 0.023 (3.45)	17.00 ± 0.058 (3.55)
Neutral detergent fibre (%)	70.00 ± 0.208 (10.57)	73.20 ± 0.006 (10.91)	73.20 ± 0.462 (10.17)	84.60 ± 0.115 (11.57)	60.00 ± 0.462 (13.92)	74.40 ± 0.462 (16.59)	75.20 ± 0.462 (16.17)	86.00 ± 0.577 (17.97)
Acid detergent fibre (%)	17.20 ± 0.115 (2.60)	19.20 ± 0.115 (2.86)	19.50 ± 0.173 (2.71)	20.40 ± 0.462 (2.79)	13.60 ± 0.231 (3.16)	14.40 ± 0.231 (3.21)	16.60 ± 0.115 (3.57)	18.40 ± 0.260 (3.85)
Hemicellulose (%)	52.80 ± 0.346 (7.97)	54.00 ± 0.148 (8.05)	53.70 ± 0.289 (7.46)	64.20 ± 0.416 (8.80)	46.40 ± 0.230 (10.76)	60.00 ± 0.683 (13.38)	58.60 ± 0.601 (12.60)	67.60 ± 0.742 (14.13)
Indigestible (%)	64.7	66.1	66.6	77.7	59.7	75.1	74.5	84.6

Figures in the parenthesis indicate wt. in mg/kernel pericarp

Dietary fibre in pericarp: The data relating to the various components of dietary fibre of normal and Opaque-2 pericarp are given in Table 2. The CF, NDF and ADF contents increased gradually from 25 days after pollination to maturity in both normal and Opaque-2 pericarp. In normal pericarp, CF content was found to be 1.40, 3.02, 3.11 and 3.50 per cent higher than in case of Opaque-2 pericarp after 25, 30,35 days of pollination respectively and at maturity. Marked difference in NDF content was not observed between normal and Opaque-2 pericarp at various stages of kernel development except at 25-day stage at which Opaque-2 contained 10 per cent higher NDF. The ADF was, however, found to be 4.6,4.8,2.9 and 2.0 per cent higher in Opaque-2 than normal at 25,30,35 days respectively and at maturity.

In normal pericarp, hemicellulose was found to be 6.0, 4.9 and 3.4 per cent higher at 30, 35 days after pollination respectively and at mature stage, except at 25 days stage at which hemicellulose content in Opaque-2 pericarp was 6.4 per cent higher. Hemicellulose content increased gradually from 25-day stage to maturity in Opaque-2 and normal pericarp; however, the differences between 30 and 35 day stage in both varieties were not well marked.

Dietary fibre in endosperm: Data on dietary fibre of normal and Opaque-2 maize endosperm are given in Table 3. In both Opaque-2 and normal endosperm, CF content increased gradually during development. The NDF and hemicellulose showed varied behaviour in Opaque-2 and in normal endosperm. In Opaque-2, both NDF and hemicellulose increased from 25 day to 35 day stage while in normal both NDF and hemicellulose increased upto 30–day stage followed by a decline. The variation in the hemicellulose level at various stages of kernel development was more marked than that of NDF. Relatively small difference in ADF content was observed between normal and Opaque-2 endosperm at various stages of development.

Dietary fibre in whole kernel: Table 4 records data on dietary fibre of normal and Opaque-2 kernel. Both in Opaque-2 and normal kernel, CF content decreased gradually during development. The decrease in CF content was more pronounced from 35-day stage to maturity, being 2.50 to 1.68 per cent in Opaque-2 and 3.00 to 2.10 per cent in normal variety. The CF content was found to be 0.20, 0.51, 0.50 and 0.42 per cent higher in normal than in Opaque-2 kernel, but NDF content was found 8.06, 16.30, 19.52 and 14.25 per cent to be higher, while hemicellulose was found to be 8.65, 16.86, 27.20 and 15.25 per cent higher in Opaque-2 than in normal kernel at 25, 30, 35 day stage respectively and at maturity.

In Opaque-2 kernels NDF, ADF and hemicellulose content increased from 25-day to 35-day stage followed by a decrease. In normal kernel ADF content increased up to 35-day stage while NDF and hemicellulose increased upto 30-day stage, followed by a decrease. It is interesting that Opaque-2 contained nearly two fold NDF and hemicellulose which substantially lowered levels of ADF than normal at all stages of development. Same trend in these fibres were also observed on weight per kernel basis.

TABLE 3. DIETARY FIBRE IN DEVELOPING NORMAL AND OPAQUE-2 MAIZE ENDOSPERM

Type of fibre	Shakti (Opaque-2)				Ganga-5 (normal)			
	25 days	30 days	35 days	Maturity	25 days	30 days	35 days	Maturity
Crude fibre (%)	0.24	0.29	0.30	0.33	0.25	0.45	0.45	0.60
	± 0.03	± 0.05	± 0.03	± 0.07	± 0.03	± 0.03	± 0.09	± 0.06
	(0.23)	(0.29)	(0.31)	(0.55)	(0.24)	(0.72)	(0.75)	(1.10)
Neutral detergent fibre (%)	9.15	13.10	14.26	12.23	7.00	10.65	9.05	7.50
	± 0.62	± 0.52	± 0.50	± 0.49	± 0.70	± 0.03	± 0.29	± 1.04
	(8.56)	(13.10)	(14.55)	(20.30)	(16.55)	(17.13)	(14.99)	(13.20)
Acid detergent fibre (%)	0.55	0.75	0.80	0.70	0.65	0.65	0.60	0.80
	± 0.03	± 0.05	± 0.06	± 0.06	± 0.03	± 0.09	± 0.06	± 0.12
	(0.51)	(0.75)	(0.82)	(1.16)	(0.64)	(1.05)	(0.99)	(1.41)
Hemicellulose (%)	8.60	12.35	13.47	11.53	6.35	10.00	8.45	6.70
	± 0.65	± 0.52	± 0.55	± 0.55	± 0.73	± 0.06	± 0.34	± 0.96
	(8.05)	(12.35)	(16.16)	(19.14)	(5.95)	(16.08)	(13.99)	(11.79)
Indigestible (%)	8.85	12.64	13.80	11.86	6.60	10.45	8.90	7.30

Figures in the parenthesis indicate wt. in mg/kernel endosperm

TABLE 4. DIETARY FIBRE IN DEVELOPING NORMAL AND OPAQUE-2 MAIZE WHOLE KERNEL

Type of fibre	Shakti (Opaque-2)				Ganga-5 (normal)			
	25 days	30 days	35 days	Maturity	25 days	30 days	35 days	Maturity
Crude fibre (%)	3.10 ± 0.058 (4.65)	2.74 ± 0.019 (4.68)	2.50 ± 0.012 (4.50)	1.68 ± 0.023 (3.27)	3.30 ± 0.012 (6.12)	3.25 ± 0.029 (7.09)	3.00 ± 0.115 (6.72)	2.10 ± 0.058 (4.71)
Neutral detergent fibre (%)	18.80 ± 0.058 (28.20)	31.80 ± 0.231 (54.31)	34.60 ± 0.058 (62.28)	29.30 ± 0.173 (57.43)	10.86 ± 1.016 (19.24)	15.50 ± 0.183 (33.79)	15.08 ± 0.072 (33.77)	14.05 ± 0.075 (31.50)
Acid detergent fibre (%)	1.48 ± 0.040 (2.20)	1.64 ± 0.231 (2.80)	2.70 ± 0.058 (4.86)	2.10 ± 0.245 (4.12)	2.10 ± 0.520 (3.90)	2.29 ± 0.115 (4.80)	2.40 ± 0.115 (5.38)	2.10 ± 0.153 (4.71)
Hemicellulose (%)	17.32 ± 0.095 (25.98)	30.16 ± 0.243 (51.31)	31.90 ± 0.058 (57.42)	27.20 ± 0.300 (53.31)	8.76 ± 0.880 (15.34)	13.30 ± 0.141 (28.99)	12.68 ± 0.167 (28.40)	11.95 ± 0.501 (26.79)
Indigestible (%)	20.40	32.90	34.40	28.80	12.10	16.60	15.70	14.10
Protein (%)	12.69	—	11.69	11.69	13.44	—	12.50	11.81
True digestibility (%)*	80.00 ± 4.4	—	84.40 ± 2.3	95.20 ± 0.9	76.30 ± 2.3	—	81.90 ± 3.5	92.80 ± 0.9

*Values from the previous study[6]. Figures in the parenthesis indicate wt in mg/kernel.

Discussion

CF and hemicellulose are the two important dietary fibre constituents which influence the utilization of various nutrients namely protein, micronutrients, etc[11,12]. The content of the dietary fibre, however, varies considerably in different varieties as well as in different stages of kernel development and in different kernel fractions. The CF and hemicellulose contents showed considerable variation in the pericarp, endosperm and in the whole kernel in normal and Opaque-2 varieties. Rice bran is also known to be rich in CF and NDF content than its endosperm[13]. The relationship between TD and CF consistantly showed a negative trend while IDC and hemicellulose showed a positive trend until 35-day stage after which the relationship tended to be negative (Fig 1). Earlier studies[14,15] also revealed that protein sources rich in CF, had lower TD values than protein with lower CF content. Variation in different stages of kernel development was particularly marked for hemicellulose content. This was particularly apparent in 'Shakti-opaque-2' composite.

Correlation coefficients between TD of whole kernel and pericarp, CF, HC, IDC and protein content in whole kernel, endosperm and pericarp are presented in Table 5, which reveals that TD of the whole kernel was signi-

TABLE 5. CORRELATION COEFFICIENT OF FIBRE CONSTITUENTS AND TRUE DIGESTIBILITY (TD) OF MAIZE

Characters (%)		Correlation coefficient
Whole kernel	**Whole kernel**	
TD	Pericarp	-0.76**
TD	Crude fibre	-0-89**
TD	Hemicellulose	0.47
TD	Indigestible content	0.35
Protein	,,	-0-67*
TD	Protein	-0.85**
Whole kernel	**Endosperm**	
TD	Crude fibre	0.56
TD	Hemicellulose	0.31
TD	Indigestible content	0.34
Whole kernel	**Pericarp**	
TD	Crude fibre	0.40
TD	Hemicellulose	0.91**
TD	Indigestible content	0.86**

*Significant; **highly significant

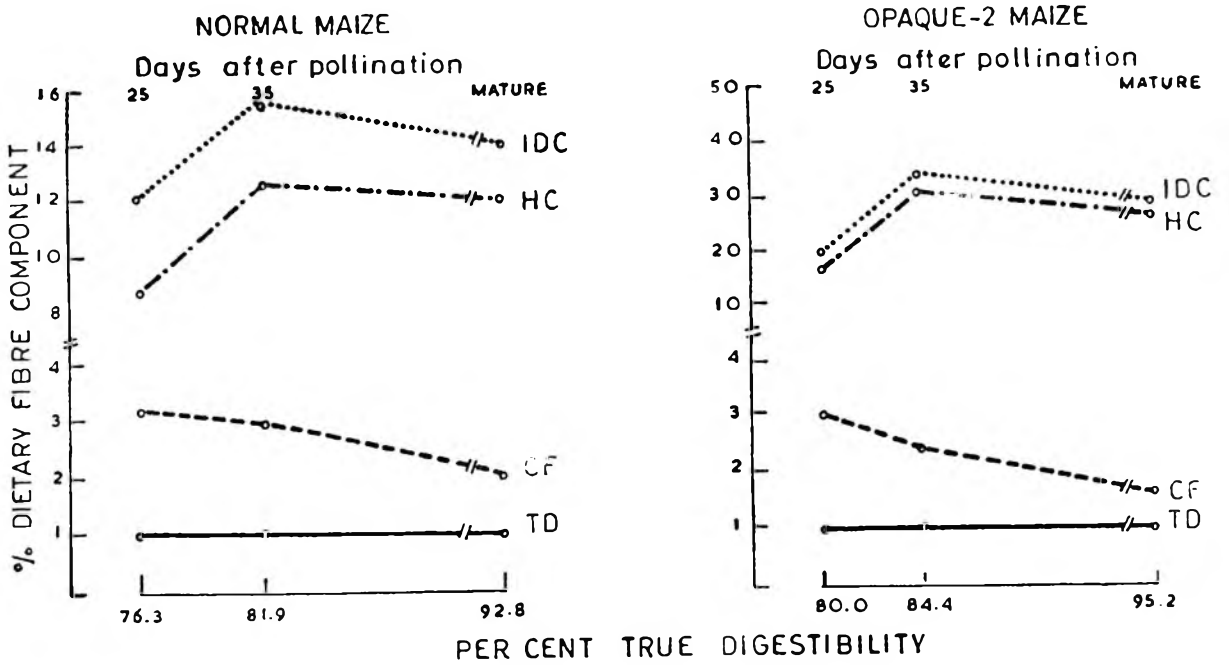

Fig. 1. Relationship between true digestibility (TD) and dietary fibre component in maize.

ficantly but negatively associated with protein, CF and pericarp content. At the pericarp level however, the association of TD with HC and IDC was positive and highly significant. None of these correlations were however, found significant at the endosperm level, thus suggesting the important role of hemicellulose of pericarp in influencing TD. Thus lower level of TD at earlier stages of kernel development may have been due to various factors of which the dietary fibre appears to be an important one. The IDC content of Opaque-2 was substantially higher than normal at all stages of kernel development, but the major component was the hemicellulose which is relatively more digestible than cellulose[16]. Moreover the embryo fraction of Opaque-2 was larger than that for normal maize. It will be interesting to determine the TD of two varieties when their pericarp are removed. Schaller[17] has also reported 89 per cent dietary fibre in maize bran. Equally of interest would be to determine the TD of two strains for their endosperms separately.

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Effect of Application of Phorate on Trypsin Inhibitor in Potato

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The trypsin inhibitor units (TIU) observed after 70 days of potato sowing were 63.75 in leaf, 11.25 in stem and 22.50 in tubers in the untreated plots; the TIU declined to zero after 80 and 90 days of sowing in leaf and stem, but in tubers it rose to 189.00 and declined to 84.00 at harvest. Similar trend was observed when phorate was applied at planting and earthing up. Free tyrosine increased from 62.50 to 99.00 μM/g sample with the advancement of potato tuber development in the control, whereas the increase was from 68.00 to 103.00 μM/g in phorate treated tubers. No significant differences were observed in different types of appliction of phorate. Protein content did not show much difference in treated and untreated samples.

Insecticides like phorate (Thimet) have been used in potato cultivation to protect the foliage from sap sucking insects as soon as they appeared. This is done by placing the insecticides either in the furrow with the seed tuber or by coating the tuber with it[1].

The insecticides which are enzymatic inhibitors, when applied to soil, may affect the microorganisms in soil[2]. Hacskaylo *et al*[3]. found reduction in soluble protein nitrogen in young cotton plants, with the increase in chloroform soluble and insoluble phosphates caused by the application of phorate in soil. Of the major proteolytic digestive enzymes in animals, tyrpsin, chymotrypsin and carboxypeptidases have been reported to be inhibited by purified proteins or polypeptides from potato tuber tissues[4]. Their presence indicates a possible storage role of inhibitor protein[5]. The inhibitor proteins are not confined to storage organs but are also found in other tissues, sprouts, roots, stems and leaves of plant and their presence in all the cases is apparently transitory. Nothing is known of the interaction between the protease and the inhibitor *in vivo*, but in view of the importance of protease in the breakdown of storage proteins in protein bodies, it is likely that it has a function in regulating the activity of the enzymes in dormant potatoes[6]. Our understanding of such biological changes in plants is scanty and hence the effect of phorate on trypsin inhibitor in potato was investigated.

Materials and Methods

Plots were designed in randomised manner. Potato ('Chandermukhi' var). was sown in the month of October 1978 (Winter crop). Phorate (0, 0-diethyl S-(ethylthio) methyl phosphorodithioate) was applied at the recommended doses viz. granular application at 1.5 kg a.i./ha at the time of planting (T_1); application of all the recommended dose at 1.5 kg a.i/ha at the time of earthing up (T_2) (10th Nov., 1978); application of half the recommended dose (0.75 kg a.i/ha) at the time of planting and the remaining half (0.75 kg a.i/ha) at the time of earthing up (T_3). Samples were taken after 70,80 and 90 days of sowing. Sampling was done at random. Three replicates were mixed and the composite samples were analysed. Leaf, stem and tubers were taken separately after washing with water which was removed by drying by blotting on a filter paper.

Trypsin inhibitor was extracted by the method of Gupta and Deodhar[7] and estimated according to Kakade *et al*[8]. Free tyrosine and tyrosine released during reaction were estimated at 280 nm in a Beckmann Spectrophotometer. Protein was determined by the method of Lowry *et al*[9]. Protease activity[8] was measured without adding trypsin.

Results and Discussion

Trypsin inhibitor activity and free tyrosine in different parts of the plant after 70 days of sowing is given in Table 1. In tubers the TIU was 22.50 in control and ranged

TABLE 1. EFFECT OF PHORATE ON TRYPSIN INHIBITOR ACTIVITY AND FREE TYROSINE IN POTATO AT 70 DAYS AFTER SOWING

	Trypsin inhibitor units (TIU/g)			Tyrosine (μ M/g)		
Treatment	Leaf	Stem	Tuber	Leaf	Stem	Tuber
Control	63.75	11.25	22.50	303.75	93.75	62.50
T_1	21.25	56.25	27.50	121.66	125.00	68.33
T_2	18.33	32.50	6.66	199.58	89.93	69.16
T_3	3.75	13.75	31.25	237.50	92.50	70.00

T_1, Full application of pesticides at the time of planting; T_2, Full application of pesticides at the time of earthing up; T_3, Half at the time of planting and half at the time of earthing up.

TABLE 2. EFFECT OF PHORATE ON TRYPSIN INHIBITOR ACTIVITY AND FREE TYROSINE IN POTATO AT 80 AND 90 DAYS AFTER SOWING

Treatment	TIU/g at the indicated days		Tyrosine (μ M/g) at the indicated days					
			Leaf		Stem		Tuber	
	80	90	80	90	80	90	80	90
Control	189	84	141	162	84	99	93	99
T_1	75	21	221	158	94	103	70	102
T_2	95	90	147	164	88	102	68	103
T_3	196	36	169	144	97	102	91	102

Trypsin inhibitor was not detected in leaf and stem after 80 days.

T_1, T_2 and T_3 are as under Table 1.

TABLE 3. EFFECT OF PHORATE ON PROTEIN (mg/g) CONTENT IN POTATO AFTER DIFFERENT DAYS OF SOWING

Treatment	Leaf			Stem			Tuber		
	70 days	80 days	90 days	70 days	80 days	90 days	70 days	80 days	90 days
Control	31.39	10.10	7.98	3.53	3.27	3.77	1.94	7.60	3.35
T_1	11.61	23.22	7.99	3.16	6.10	3.60	1.79	6.40	2.38
T_2	23.10	11.10	5.22	3.82	6.08	4.25	1.79	6.96	2.71
T_3	16.73	13.61	6.68	3.56	6.49	4.28	1.59	6.73	2.49

T_1, T_2 and T_3 are same as under Table 1.

from 27.50 to 31.25 in treated samples. Free tyrosine in control tubers was 62.50 μM/g whereas in phorate treated tubers it ranged from 68.33 to 70.00 μM/g depending upon the method and time of application. This may, in part be due to the effect of phorate on polyphenoloxidase enzyme system.

Trypsin inhibitor activity and free tyrosine content after 80 and 90 days of sowing are given in Table 2. There is a total elimination of TIU in leaf and stem in both the cases. This may arise from synthesis of trypsin inhibitor at a particular stage of development and getting it translocated into tubers during tuber development. Trypsin inhibitor presence in all the cases is apparently transitory indicating a possible temporary storage role[5]. TIU was maximum (189.00) in control tubers and minimum in treated samples (21.00). This reduction in trypsin inhibitor after treatment with phorate would suggest inhibitory role of phorate in the trypsin inhibitor synthesis at a particular stage of tuber development.

After 80 days of growth, free tyrosine increased in leaves and stem as compared to the control, while it decreased in tubers after treatment. At 90 days of growth (viz harvesting time) free tyrosine decreased in in the treated leaf, but increased in stem and tubers. There is, however, not much of a difference among the different types of applications. There seems to be no correlation between tyrpsin inhibitor and free tyrosine content during development of tubers. Protease activity was also absent in all the cases. Mode of action of phorate on trypsin inhibitor is not known.

Effect of phorate on soluble protein in potato is presented in Table 3. Protein content declined from 31.39 to 7.98 mg/g in leaves in case of control while the decline was from 23.10 to 5.22 mg/g in leaves of treated plants during 90 days of growth. Phorate may affect the synthesis of protein or its translocation. Protein content rose in stem and tubers in control and the treated ones. After 80 days of growth protein content is quite high in stem and tubers. But there is marked decrease in protein content after 90 days of growth (at the time of harvesting). This may reflect mobilisation of protein for conversion of proteins into carbohydrates. Phorate may be affecting some of the unknown enzymes, since phorate is a systemic insecticide mainly used for the treatment of seed at sowing for the protection of the seedlings from sap sucking insects. Besides raising the quantum of potato production, the residual effect on the crop should also be considered.

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Residues of Phorate, Carbaryl and Endosulfan in Peas *(Pisum sativum)*

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Residues of phorate in soil treated with 1.5 kg a. i/ha and in pea plants (whole, pods and grains) grown on it and foliar applications of 0.1 and 0.2 per cent carbaryl suspension and 0.05 and 0.1 per cent endosulfan emulsion in/on plants were determined. Phorate residues persisted for 90 days in soil and the absorption in the plant at any stage of growth was 30-60 per cent of that present in soil. Both carbaryl and endosulfan persisted on the plant and pods for 25 and 15 days respectively. Carbaryl residues on pods were below tolerance level in 1 to 3 days and endosulfan in 3 to 7 days. Carbaryl and endosulfan in the grains were observed 7 days after treatment and attained maximum in 15 days. The residues of the three insecticides in the grains were within the tolerance limits.

Peas suffer from the attack of pea leafminer (*Phytomyza atricornis* Meign), agromyzid stemfly (*Melangromyza phaseoli* Coq.), aphids (*Macrosiphum pisum*, Harris) and pod borer (*Etiella zinckenella* Treitschke). Soil applications of disulfoton, phorate and aldicarb for the control of sucking pests and foliar sprays of carbaryl and endosufan against pod borer are recommended[1-3]. With a view to ascertaining the safety to consumers, the residues of phorate, carbaryl and endosulfan were estimated on peas and presented here.

Materials and Methods

A crop of pea *var.* 'Bonneville' was raised during October 1978 to February 1979 at the Rajasthan College of Agriculture, Udaipur in clay loam soil, in plots measuring 5 m×4m in a randomised block design. A spacing of 50 cm between rows and 20 cm between plants was maintained. Phorate (10 per cent granules @ 1.5 kg a.i./ha) was applied to the soil in furrow below the seed before sowing. All recommended agronomic practices were followed. Carbaryl (0.1 and 0.2 per cent suspension) and endosulfan (0.05 and 1.0 per cent emulsion) at 900 l/ha were sprayed on the crop at the initiation of pods. A control was also maintained; each treatment was replicated thrice.

Samples from phorate treated soil and plants grown were collected at intervals of 15 days. Carbaryl and endosulfan treated plant samples (whole plant, green pod and grains) were collected at 0, 1, 3, 7, 15, 21 and 25 days after treatment.

Distilled chloroform, methylene chloride and *n*-hexane at the rate of 3 to 4 ml/g sample were used as solvents for extracting phorate, carbaryl and endosulfan respectively. Phorate was extracted from soil by tumbling on a motorised shaker for 30 min. The extract was filtered through a Whatman filter paper No. 1 and collected in the reagent bottle. The plant samples were chopped and macerated (with solvent @ 3 ml/g sample) in a waring blender. The plant extract was filtered through a scintered funnel which contained a thin layer of hyflosupercel and anhydrous sodium sulphate. The soil extract required no 'clean up', whereas the plant extracts were 'cleaned up' as per procedure described[4]. The residues were estimated according to the methods described for phorate[5], carbaryl[6] and endosulfan[7]. The

recoveries of phorate, carbaryl and endosulfan from the fortified soil and plant samples ranged from 90 to 95, 88, to 90 to 93 per cent respectively.

Results and Discussion

Residues of phorate in soil and plant: As a result of application of phorate (@ 1.5 a.i./ha) to the soil, the deposits were 30.22 ppm (Table 1). More than 58 per cent were reduced in 15 days and the residues were below detectable level in 90 days, showing complete dissipation. Earlier reports indicate its persistence in soil for 50 days under rape seed[8], 60 days under cauliflower[4], 75 days under potato, sweet potato and onion[9]. With the increase in dose to 3 to 4 kg a.i./ha the persistence had also increased to 70 days under mustard crops[10].

Phorate being systemic is absorbed by plant system. The results (Table 1) show that most of the phorate in the soil was absorbed upto 45 days, thus providing protection to plants. About 30-60 per cent of phorate present in the soil was absorbed by the plant. At 75 days, when the pea pods were of marketable size, the residue in the soil was only 0.57 ppm while in plant and pods it was 0.10 ppm. However, at 90 days the insecticide was not detectable in any of these materials. The grains in the pod contained no phorate and were thus safe to the consumers. The tolerance level reported is 0.5 ppm phorate[11]. The results of the present study corroborate the observations on phorate on other crops[4,9,10,12].

TABLE 1. RESIDUES OF PHORATE (PPM) IN SOIL AND IN PEA

Days after treatment	Soil	Whole plant	Pod
0	30.22	—	—
15	12.60 (58.30)	4.13 (13.66)	—
30	11.12 (63.20)	5.47 (18.10)	—
45	4.98 (83.52)	3.07 (10.16)	—
60	1.00 (96.69)	0.58 (1.92)	—
75	0.57 (98.11)	0.10 (0.33)	0.10 (0.33)
90	BDL (100.00)	BDL (100.00)	BDL (0.0)

Average of three replicates: BDL - Below detectable level; data in parentheses represent percentage reduction in soil and absorption in plant samples. No residue was detcted in grain.

TABLE 2. RESIDUES OF CARBARYL (PPM) IN PEA

Days after treatment	Whole plant 0.1% level	Whole plant 0.2% level	Green pod 0.1% level	Green pod 0.2% level	Grain 0.1% level	Grain 0.2% level
0	12.75	31.20	5.48	10.73	—	—
1	11.56 (9.33)	27.27 (12.59)	2.33 (57.48)	6.15 (42.68)	—	—
3	7.92 (37.88)	20.32 (34.87)	1.20 (78.10)	2.08 (80.61)	—	—
7	3.43 (73.09)	11.67 (62.60)	0.48 (91.24)	0.63 (94.13)	0.47	0.57
15	1.55 (87.84)	6.53 (79.09)	BDL (100.00)	BDL (100.00	1.20	1.20
21	0.62 (95.14)	1.45 (95.35)	—	—	BDL	BDL
25	BDL (100.00)	BDL (100.00)				

Average of three replicates; BDL-Below detectable level; data in parentheses represent percentage reduction.

Residues of carbaryl: The application of 0.1 and 0.2 per cent suspension resulted in the initial deposits of 12.75 and 31.20 ppm respectively on whole plant and 5.48 and 10.73 ppm respectively on pods (Table 2). The residues of both the insecticides reached below detectable level in 25 days in plants and 15 days on pods. The residues on pods was within the tolerance level of 5 ppm[13] after 1 day as against 3 days at the higher dose. These findings corroborate earlier observations on long melon[14], maize[15] and grapes[16] but not on tomato[17] and cowpea[18] perhaps on account of the higher application doses.

Carbaryl residue of 0.47 and 0.57 ppm were detected for 0.1 and 0.2% level of application respectively 7 days after application on the pods, which further increased to 1.20 ppm after 15 days. However, after some days, the residues were below the detectable level or nil. Though the quantity transolcated to the pea grains was far below the tolerance level, the trend observed indicate that if carbaryl was used in higher doses, then these may pose threat to consumers.

Residues of endosulfan: A deposit of 20.83 ppm on whole plant and 7.16 ppm on pods was observed on application of 0.05 per cent endosulfan emulsion. Doubling the dose of application also resulted in two times increase in residues on plants and pods (Table 3). The residues on whole plants and green pods reached below detectable level in 25 and 15 days respectively. Similar results were also obtained earlier on Bengal

TABLE 3. RESIDUES OF ENDOSULFAN (PPM) IN PEA

Days after teatment	Whole plant		Green pod		Grain	
	0.05% level	0.1% level	0.05% level	0.1% level	0.05% level	0.1% level
0	20.83	41.66	7.16	15.75	—	—
1	16.68 (19.92)	33.13 (20.47)	4.12 (42.46)	11.20 (28.89)	—	—
3	11.70 (43.83)	22.13 (46.88)	1.69 (76.39)	4.35 (72.38)	0.49	0.65
7	4.58 (70.01)	7.08 (83.00)	0.36 (95.97)	0.44 (97.20)	1.58	1.72
15	2.33 (88.81)	3.58 (91.40)	BDL (100.00)	BDL (100.00)	0.67	0.67
21	0.49 (97.64)	1.84 (95.58)	—	—	BDL	BDL
25	BDL (100.00)	BDL (100.00)	—	—	—	—

Average of three replicates; BDL-Below detectable level.
Data in parentheses represent percentage reduction

gram[19] and soyabean[12]. The residues on green pods attained the level of below tolerance (2 ppm) in 3 days in lower concentration and 7 days in higher concentration[13]. However, slightly higher intervals of 5 and 10 days were reported earlier on pea pods[20].

Endosulfan was also found in the grains, to the extent of 0.49 to 0.65 ppm 3 days after application. The residue levels increased to 1.58 and 1.72 ppm in 0.05 and 0.1 per cent concentrations in 7 days. However, it got reduced to 0.67 ppm and below detectable level in 15 and 21 days of treatment respectively. These results also indicate that endosulfan should not be used on pea, when the crop is nearing harvest or at higher than the recommended doses. Absorption of endosulfan in grains of Bengal gram[19], soyabean pod[12] and maize grain[21] husk[22] have been reported earlier.

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Evaluation of Residues of Six Recommended Insecticides on Cotton

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Residues of DDT, HCH, endosulfan, fenitrothion, quinalphos and carbaryl were estimated in cottonseed and lint collected from supervised trial during 1978. Endosulfan, fenitrothion, quinalphos and carbaryl residues in cottonseed were quite low and offered no problem despite their six applications during the flowering and boll formation stage. However, six sprays of DDT at a fortnightly interval beyond the vegetative phase resulted in build-up of its residues. The general high level of insecticide residues found in cellulose-rich cotton lint also needs to be viewed with concern.

Cotton crop requires intensive sprays of insecticides even under the recently recommended integrated pest control programme. Though cotton is primarily grown for fibres cottonseed also constitutes an important source of cooking oil and animal feed meal. The need for proper evaluation of the residues of recommended insecticides, therefore, becomes imperative.

Of the six insecticides evaluated, some information is already available on the residues of DDT,[1,2] endosulfan[3] and quinalphos[4] on cottonseed. None of these insecticides left excessive residues when normal sprays were carried out. Reviewing the situation, however, JMPR[3] observed that the extent of residues on cottonseed and lint would depend upon the frequency and dosage applied and per cent boll opening at the time of application. Since the recommended spray schedule even in the integrated pest control programme consists of at least six sprays of any of the insecticides -carbaryl, DDT, endosulfan, fenitrothion and quinalphos, continuing upto the boll maturation stage in addition to the two applications of DDT and HCH mixture in the vegetative phase, the present communication reports the residue status of these insecticides on cottonseed and lint collected a fortnight after the final spray.

Materials and Methods

Field experiment: Early maturing variety of cotton (*Gossypium hirsutum* L.) was grown during May to October 1978 at the University farm according to the recommended practices[5]. The experimental field measuring 650 sq.m. was demarcated into 6 equal plots of 30 × 3.5m each having 5 rows of the crop 0.7 m apart. The crop in the 5 plots was sprayed with DDT and benzene hexachloride (HCH) (50% WP) in a mixture @ 0.250 kg active ingredient (a.i.) acre each, using knapsack sprayer on 6th and 21st July, 1978 adopting the criteria of 50% jassid injury to the leaf. The 6th untreated plot was sprayed with an equivalent quantity of water.

After 15 days, at the initiation of flowering, each one of the DDT and HCH treated plots was sprayed 6 times with one of following five insecticides at a fortnightly interval for the control of pink bollworm:

Insecticide	Formulation	Dosage (a.i./acre)	Concn of spray fluid (%)
1. DDT	50% w.p.	500 g.	0.25
2. Endosulfan	35% e.c.	350 g.	0.30
3. Fenitrothion	50% e.c.	425 ml.	0.40
4. Quinalphos	25% e.c.	200 ml.	0.20
5. Carbaryl	50% w.p.	500 g.	0.5

The control plot was treated with water.

Sampling: On 10th October, 15 days after the final spray, fully opened bolls were picked up separately and ginned with a hand operated ginning machine; ginning ratio was 2:1. Both lint and cottonseed were kept frozen at -18°C till extracted and this period did not exceed a week.

Analysis: Residues of insecticides in cottonseed were determined in oil and residual cake separately obtained by Soxhlet extraction in n-hexane to facilitate the analysis. The ratio of oil to cake was 4:21. The residues from the two fractions were computed on proportional weight basis representing the cottonseed. Residues of DDT and HCH from the first two sprays during the vegetative phase were carried out in samples collected from plots later treated with organophosphorus or carbamate insecticides. Samples from control plots served

for background contamination of insecticides especially DDT and HCH.

Extraction and cleanup: For the estimation of residues of DDT and HCH, oil dissolved in petroleum ether was digested with concentrated sulphuric acid (sp. gr. 1.84) by its dropwise addition in a specially designed glass column and separatory funnel apparatus[6]. The petroleum ether was withdrawn after acid digestion and washed with distilled water till neutral to litmus. The petroleum ether fraction was concentrated for GLC analysis. As the other insecticides when subjected to this acid treatment showed 29 to 55% loss, a separate method was adopted for extraction and clean up.

Samples of oil containing residues of endosulfan, fenitrothion, quinalphos and carbaryl were dissolved in n- hexane and partitioned thrice with acetonitrile in a separatory funnel. The combined acetonitrile fraction was returned to n-hexane after dilution with four volumes of water and 10 ml of sodium chloride for samples containing endosulfan, while for fenitrothion, quinalphos and carbaryl residues, the residual aqueous electrolyte-acetonitrile phase was reextracted twice with dichloromethane and combined with the n-hexane phase.

The n-hexane portion meant for endosulfan residues was concentrated to about 5 ml in a rotary vacuum evaporator and column chromatographed on activated neutral alumina equilibrated with 4% moisture. Graded elution with n-hexane and n-hexane -benzene (1:1) into 2 fractions retained DDT, HCH and two endosulfan isomers in the first with endosulfan sulfate in the second. These were concentrated to a suitable volume by vacuum rotary evaporator for analysis.

The combined n-hexane and dichloromethane phase for fenitrothion, quinalphos and carbaryl were concentrated to about 5 ml in a rotary vacuum evaporator, followed by column chromatography on activated neutral alumina equilibrated with 5% moisture. Chloroform was used for elution and the elutant concentrated to almost dryness and taken up in 10 ml of benzene for analysis.

The cottonseed cake after Soxhlet extraction was reextracted and cleaned up by the method[7] applicable to low fat and low moisture substrates after slight modifications for determination of residues of DDT, HCH and endosulfan. Twenty grams of the subsample was extracted by overnight dipping in acetonitrile-water (65:35) followed by partitioning into n-hexane after adding four volumes of water and 10 ml of saturated sodium chloride. The n-hexane fraction was concentrated to about 5 ml and chromatographed on activated neutral alumina column (4% moisture content) using graded elution as described earlier for the oil fraction.

Fenitrothion, quinalphos and carbaryl from the residual cotton seed cake were further extracted in chloroform-methanol (9:1), and the extract concentrated to about 5 ml *in vacuo* followed by column chromatography on activated neutral alumnia deactivated with 5% moisture and elution with chloroform. The eluate was concentrated *in vacuo* and taken up in acetone for analysis by GLC and TLC. The residues of the test insecticides in the lint were extracted in n-hexane+acetone (9:1) by overnight dipping and the filtrate after suitable concentration was analysed by GLC and confirmed by TLC.

Gasliquid chromatographic analysis: Packard Model (7624) having electron capture and flame ionization detectors was employed for the estimation of residues. DDT, HCH and endosulfan were analysed on a 1.84 m long and 3 mm diameter Pyrex glass column packed with 5% D.C. 200 on gas chrom Q. using electron affinity tritium source detector. For the separation of various isomers of HCH and DDT related compounds, column packed with 5% OV 17 and 5% OV 210 on gas chrom Q were also employed. The operating conditons of the organochlorine insecticides have been described[8]. The identity of the residues was confirmed by microalkali treatment technique[9] followed by electron capture GLC analysis. This treatment eliminated all isomeric peaks of HCH and resulted in shift of the peaks of p, p'-DDT, p,p'-TDE and o,p'-DDT to that of p,p'-DDE and o,p'-DDE. The two separately eluting peaks of endosulfan A and B were transformed to a single peak having an intermediate retention time while the endosulfan sulphate peak got transformed into two peaks after the treatment. The major one was eluted earlier to the retention time of endosulfan sulfate and was utilized for calculating the quantity of this metabolite.

The residues of fenitrothion and quinalphos were estimated by KCl modified flame ionization detector following the conditions described earlier[10]. These conditions permitted estimation of 'oxons' as well.

Thin-layer chromatography: The highly sensitive enzyme-inhibition technique[11] was adopted for the estimation of residues of carbaryl and confirmation of the identity of fenitrothion and quinalphos. Using goat liver as the enzyme source and indoxyl acetate as the substrate, 100 to 200 ng of these insecticides, could be detected with ease. The identity of the carbaryl residues were established by TLC using chromogenic reagent-p-nitrobenzene diazonium fluoborate after hydrolysis with methanolic-KOH[12]

For the confirmation of the identity of DDT, HCH and endosulfan residues, silver nitrate impregnated alumina-g plates were exposed to UV-light after developing[13].

The methodology used for residue analysis of test insecticides gave satisfactory recovery ranging from 69 to 79 per cent and 81 to 84 per cent in fortified samples

Table 1. Residues of insecticides in cotton sprayed during vegetative and boll formation stages

Insecticide	No. of sprays	Type of residue	Residues (ppm)	
			Cottonseed	Lint
DDT	8	p,p'-DDT	1.70	3.60
		o,p'-DDT	0.64	0.90
		p,p'-DDE	0.19	0.11
		p,p'-TDE	0.06	0.30
Endosulfan	6	Alpha	0.22	0.33
		Beta	0.25	0.70
		Sulfate	a	0.40
Fenitrothion	6	P C	0.06	0.27
Quinalphos	6	P C	0.16	0.50
Carbaryl	6	P C	0.22	0.08

a = not detected PC = parent compound.

Table 2. Residues of DDT and HCH in cotton Sprayed during vegetative phase

Insecticide	No. of sprays	Type of residues	Residues (ppm)	
			Cotton seed	Lint
DDT	2	p,p'-DDT	0.34	0.59
		o,p'-DDT	Traces	Traces
		p,p'-DDE	0.03	0.08
		p,p'-TDE	Traces	Traces
HCH	2	alpha	0.043	0.010
		beta	—	—
		gamma	0.063	0.013
		delta	—	—
DDT*	—	p,p'-DDT	0.32	0.41
		o,p'-DDT	0.03	0.22
		p,p'-DDE	—	—
		p,p'-TDE	0.01	0.08
		Total	0.36	0.71
HCH*	—	alpha	0.011	0.010
		beta	—	—
		gamma	0.014	0.010
		delta	—	—
		Total	0.025	0.020

* unsprayed during vegetative phase.
– Not detected.

of cottonseed (oil and cake basis) and lint respectively. However, the results have been presented without correction for recovery.

Results

The residue data of the insecticides sprayed during vegetative as well as flowering and boll formation stage are given in Table 1 and the weather conditions in the Appendix. Cottonseed and lint were found to be contaminated with the residues of the insecticides. Lint contained higher quantitities of residues than the cottonseed. Maximum residues were left by DDT while the residues of fenitrothion and carbaryl were low.

The residues of DDT were mainly found as p,p'-DDT and o,p'-DDT as in technical DDT. The proportion of metabolites-p,p'-DDE and p,p'-TDE present was very low. Endosulfan residues were constituted by its alpha and beta isomers though small but measurable quantitites of endosulfan sulfate were present in lint only. No detectable metabolites of carbaryl, fenitrothion and quinalphos were found in cottonseed or lint.

DDT and HCH left 0.37 and 0.67 ppm and 0.106 and 0.023 ppm residues in cottonseed and lint respectively when sprayed during the vegetative phase (Table 2). DDT residues were mainly detectable as p,p'-DDT with small quantity of p,p'-DDE while HCH residues were present as its alpha and gamma isomers. Similar level and pattern of DDE and HCH were found in cottonseed and lint, even in the crop not sprayed with insecticides, except for slightly lower level of HCH in cottonseed.

Discussion

The maximum level of DDT residues in cottonseed and lint (Table 1) is indicative of the progressive build-up of this persistent insecticide through repeated sprays continuing upto boll maturation stage and having rate of loss not commensurate with the application. The long persistence of DDT leading to its accumulation in various components of the environment has been sufficiently documented[14]. The level of DDT residues found was much higher than that observed from five applications at 3-weekly intervals in Sudan[1]. The mean level of 0.491 ppm DDT reported in cottonseed is comparable to 0.36 ppm DDT residues in the present studies resulting from its two applications made during the vegetative phase (Table 2). El-Zorgani (1979, personal communication) has attributed the higher DDT residues to the contribution of six sprays during flowering and boll formation stage in early maturing cotton variety and also to higher recoveries of the pesticide by the improved analytical method.

The maximum level of DDT residues reported was 0.232 ppm in cottonseed samples collected from various field experiments carried out in different parts of India during 1976[2]. In these experiments, DDT had been sprayed 4-12 times at 0.25 per cent concentration and residues analysed colorimetrically. However, these results lack necessary experimental details for making meaningful comparisons.

APPENDIX: WEATHER CONDITIONS DURING THE EXPERIMENTAL PERIOD OF THE YEAR 1978

Month	Temperature(°C)			Relative humidity (%)			Rainfall
	Min.	Max.	Mean	Min.	Max	Mean	(mm)
May	23.3	42.3	32.8	15	43	29	25.7
June	25.4	37.9	31.7	42	66	54	146.1
July	25.4	33.2	29.3	70	84	77	213.9
August	25.0	33.0	29.0	69	89	79	178.8
September	22.3	33.4	27.9	55	88	73	429
October	15.7	32.1	23.9	31	89	60	55

Figures are the mean for the month.

The occurrence of even higher insecticide residues in lint seems to be the direct fall of the spray on the bolls and their holding on the cellulose-rich fibre. The contamination of cottonseed is rather a secondary transfer from the lint. Analysis of a popular brand of sterilized cotton lint also showed 0.4 ppm of DDT residues. Spraying the crop with DDT and HCH before the onset of flowering resulted in much lower residues in cottonseed and lint (Table 2) which were similar to the level occurring in the untreated crop. These results point out to the environmental contamination of cottonseed and lint with these insecticides akin to the contamination of soils[8], cereals[15], animal feed[16], milk and milk products[17]. The relatively lower magnitude of HCH residues in lint may be due to its two sprays in the vegetative phase only. The occurrence of endosulfan sulfate in cotton lint in addition to its alpha and beta isomers could be attributed to the photoconversion of the insecticide as a result of its exposure to light as reported earlier[18,19].

The general level of low residues of endosulfan, fenitrothion, quinalphos and carbaryl in cottonseed and lint from the repetitively treated crop and their non-occurrence in the untreated crop shows their fast rate of disappearance as also their lack of persistence potential associated with environmental contamination. The residues of endosulfan, carbaryl[20] and quinalphos[4] in cottonseed were even below the prescribed maximum residue limits of 0.5, 1.0 and 0.25 ppm respectively. Further research is necessary for assessing the need of application of DDT during the flowering and boll formation stage which resulted in high residues.

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RESEARCH NOTES

PRESSOR AMINES IN FOODS

Analysis of some of the commonly consumed food commodities of India revealed that they have very low levels of histamine, serotonin and tyramine and therefore, cannot be considered as substantial sources of these amines even when consumed at levels above the usual dietary levels.

Therapy with monoamineoxidase inhibitors is known to result in accumulation of monoamines like tyramine, leading to many undesirable effects[1-3]. Meals rich in cheese, yeast products or broadbeans have been reported to lead to attacks of forceful heart beat, severe headache and hypertension because of the high levels of tyramine content[4]. Thus, with a view to find out the potential food sources of the common pressor anines like tyramine, serotonin, and histamine, the present work was undertaken.

Histamine content of various foods was estimated according to the method of Taylor *et al*[5]. Serotonin was estimated by Bogdanski's method[6] and tyramine by the method of Udenfriend *et al*[7].

Histamine, serotonin, and tyramine contents of some of the food commodities are presented in Table 1. It was observed that among the legumes studied, white cowpea (*Vigna catiang*) had the highest histamine content of 9.5 μg/g fresh sample, while in Bengal gram the content was lowest (0.1 μg/g). Black gram, field bean, soybean, green gram and white pea contained between 2.5 and 4.3 μg/g. Among the cereals tested, bajra was the richest followed by jowar, wheat and rice, the range being 2.8-5.7 μg/g. Dry prawn had as low as 0.2 μg/g histamine as against 6.5 μg/g in dry Bombay duck. In case of tomato, ripening appreciably increased histamine. Curd contained 1μg/g of histamine.

Serotonin was found to be absent in Bengal gram flour, rice, unripe tomato, and apple. Unripe banana was rich quite in serotonin, which was found to decrease appreciably on ripening. Soybean had 5.8 μg/g of serotonin as against other cereals and legumes which ranged between 0.5 and 2.2 μg/g. Tyramine was absent from white pea, dry prawn, unripe banana and apple. Ripe tomato and guava had around 3 μg/g. tyramine. Bajra, rice, dry Bombay duck, ripe banana ranged around 2.0-2.6 μg/g, while Bengal gram, field bean, black gram, raw tomato and curd had between 1.2 and 1.9 μg/g tyramine. There is no comparable data available as yet on any of these commodities. The levels of these amines as observed in Indian cheese are however, very low as compared to those for Camembert and other cheeses[8,9].

TABLE 1. AMINE CONTENTS OF VARIOUS FOOD COMMODITIES

Food Commodity	Histamine (μg/g)	Serotonin (μg/g)	Tyramine (μg/g)
Cereals			
Whole wheat flour	3.5	0.5	0.3
Whole jowar flour	4.2	1.0	0.4
Whole bajra flour	5.7	2.2	2.6
Rice flour	2.8	0.0	2.5
Legumes			
Whole soybean flour	2.4	5.8	0.4
Whole white peaflour	4.3	0.6	0.0
Whole cowpea (red) flour (*Vigna sinensis*)	6.4	1.2	0.4
Whole Cowpea (white) flour (*Vigna catiang*)	9.5	1.7	0.0
Whole black gram flour	2.5	1.7	1.4
Field bean dal flour	3.1	0.9	1.6
Whole green gram flour	2.6	0.8	0.7
Whole Bengal gram flour	0.1	0.0	1.3
Fish			
Dehydrated Bombay Duck	6.5	3.5	2.6
Dehydrated prawn	0.2	6.5	0.0
Vegetables			
Ripe tomato	11.1	4.7	3.0
Raw tomato	0.5	0.0	1.2
Fruits			
Ripe banana	0.0	1.1	2.1
Raw banana	0.0	5.9	0.0
Apple	0.7	0.0	0.0
Guava	0.0	0.4	3.6
Dairy Products			
Cheese	0.0	1.4	1.1
Curd	1.0	0.3	1.9

Values expressed on fresh weight basis; all values are average of four determinations.

The present studies have therefore, revealed that all the commodities analysed here contained the three major amines at such low levels that they cannot be considered as hazardous sources[10,11] of these amines, even when consumed at levels well above the usual dietary levels.

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IDENTIFICATION AND ESTIMATION OF SOME VOLATILE CARBONYLS IN THREE TYPES OF KHOA BY GLC

The relative proportions of steam volatile carbonyls isolated from fresh samples of the three types of khoa (from cow's milk, buffalo's milk and khoa purchased from market) revealed that pentan-2-one was the major volatile carbonyl component in khoa samples from cow's milk (11.67%) and buffalo's milk (7.15%), while heptan-2-one was identified to be the major carbonyl component in market khoa (42.15%). In all, 9 different carbonyls were identified in each of the three types of khoa samples.

Khoa is an open pan desiccated whole/(to a very small extent) skimmed milk; nearly 7 per cent of the total milk produced in India is converted into Khoa[1]. This desiccated part of all milk solids with 25-35% moisture forms the base for a variety of Indian confections like *pera*, *burfi*, *gulabjamun*, *mohandas* and *carrot halva*. The quality of the base material largely decides the acceptance of the end products. The flavour of khoa significantly influences the quality characteristics of the product and in turn affects the acceptance of sweetmeats by the consumers. Bolding and Taylor[2] reported the presence of C8 to C14 homologous series in heated milk fat. Hemavathy and Prabhakar[3] observed the presence of methyl ketones and saturated aldehydes (C4, C5, C6, C8 to C10 and C12) in khoa. They found that khoa was rich in lower carbon chain length methyl ketones and saturated aldehydes, but the type of milk used has not been mentioned. In this paper we have dealt with the steam volatile carbonyls in the three types of fresh khoa samples prepared in the laboratory from cow's and buffalo's milks and the one procured from market.

Khoa from cow and buffalo milks were prepared using fresh milk collected from the Experimental Dairy, National Dairy Research Institute, Karnal. The cow's milk was from a herd of cross bred and Indian cow's while buffalo's milk was from a Murrah herd. For the production of khoa from cow milk, it was standardized to 4.5% fat and 8.5% solids-not-fat (SNF), while buffalo milk was standardized to 6% fat and 9% SNF. The method standardized by De and Ray[4] was followed for the preparation of khoa. The market samples of khoa were collected from the nearby market ensuring that the product was freshly manufactured. The samples were brought from the market well wrapped in *Dhak* leaves and the bulk placed in a bamboo basket.

The volatile carbonyls in khoa were determined using gas liquid chromatography (GLC). The solvents were made carbonyl free by the procedure of Schwartz and Parks[5]. 2,4-Dinitrophenylhydrazine (DNPH) reagent was prepared by dissolving DNPH (1.2 g) in 2N HCl (300 ml). 2,4-Dinitrophenylhydrazones (DNPs) of pure alkan-2-ones (C3, C5, C7, C8, C9), alkanals (C1, C2, C3, C4) and benzaldehyde were prepared[6] and checked by melting point data. GLC analysis was performed on "Varian Aerograph Model 1968-4" flame ionization gas chromatograph.

Khoa (300 g) was steam distilled and the distillate (1 l.) was trapped in the DNPH reagent (300 ml). The distillate was allowed to stand overnight and the precipitated DNPs were collected by filtration under suction. The DNPs were washed several times with glass distilled water before extracting with chloroform (carbonyl-free) and subsequently the chloroform was removed to obtain DNPs.

The carbonyl-DNPs were separated into individual components by following the procedure of Fedeli and Cirimeli[7]. The carbonyl DNPs (approximately 10 mg) of each sample were dissolved in chloroform (0.3 ml) and injected (2 μl) directly into the gas chromatograph using stainless steel column ($5' \times \frac{1}{8}''$) packed with SE 30 (3%, on Chromosorb W. Nitrogen) at a constant pressure of 21 psig was used as the carrier gas. The temperature of the injection port was 270°C and the column temperatures were between 166° and 250°C. Initial isothermal period was 5 min. and the programming was done at the rate of 6° per min. Few peaks could be identified by comparative GLC of the authentic carbonyl-DNPs carried out strictly under the identical conditions. The relative percentages of the carbonyl-DNPs of the samples were calculated by triangulation.

The data on the relative proportions (%) of the corbonyls in khoa samples indicate (Table, and Figure) that (i) in fresh samples of cow milk khoa, the minimum value was for benzaldehyde (0.87), and the maximum was for

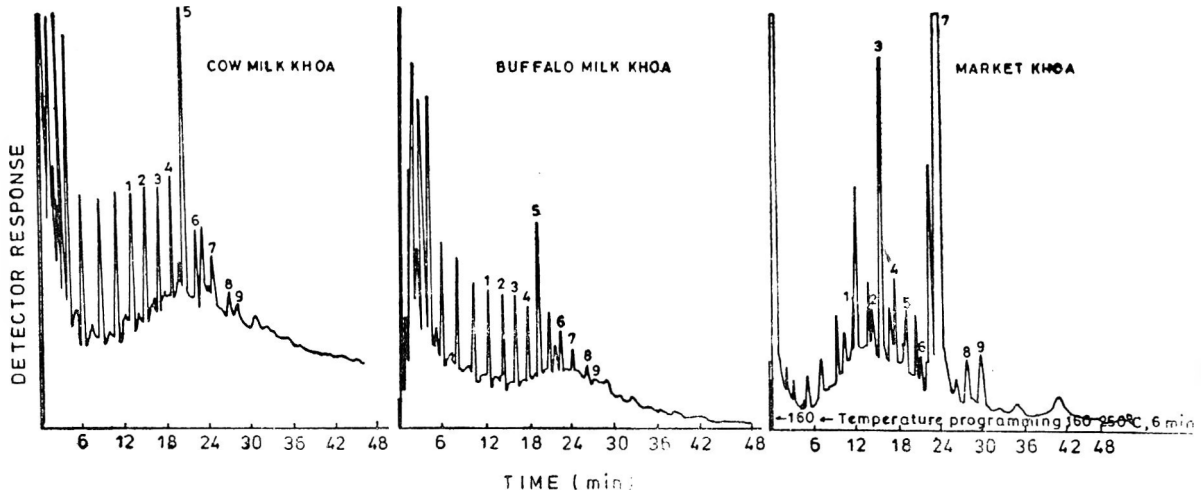

Fig. GLC Separation of 2-4, DNPs of volatile carbonyls from fresh cow milk Khoa, buffalo milk Khoa and market Khoa.

pentan-2-one (11.67) among the identified carbonyls, while the unidentified carbonyls accounted for 68.46%; (*ii*) in fresh samples of buffalo milk khoa, again the minimum value was for benzaldehyde (0.50), and the maximum was for pentan-2-one (7.15) among the identified carbonyls, while the unidentified carbonyls remained 73.33%, a little more than in the case of fresh samples from cow milk khoa; (iii) similarly, in fresh market samples the minimum was for benzaldehyde (0.21), while the maximum was heptan-2-one (42.15) among the identified carbonyls, which accounted for 65.23%.

TABLE. CARBONYL FRACTIONS IDENTIFIED FROM GLC PEAKS OF KHOA FROM THE LABORATORY AND MARKET SAMPLES

No. of C atoms and compound 2:4-dinitrophenyl hydrazones of:	Cow milk khoa	Baffalo milk khoa	Market khoa
Formaldehyde (1)	4.43	4.73	3.01
Acetaldehyde (2)	4.43	4.07	1.44
Propionaldehyde and/or Acetone (3)	2.82	3.42	6.45
Butyraldehyde (4)	3.94	2.75	1.93
Pentan-2-one (5)	11.67	7.15	3.37
Heptan-2-one (6)	2.00	1.05	42.15
Benzaldehyde* (9)	0.87	0.50	0.21
Octan-2-one (7)	0.26	1.50	2.30
Nonan-2-one (8)	1.12	1.50	4.37
Total identified carbonyls	31.54	26.67	65.23
Total unidentified carbonyls	68.46	73.33	34.77

Values in the parantheses are the peak numbers as shown in Fig.
*Identified tentatively.

It can be obscrved that the proportion of heptan-2-one in the market sample was much more (42.15%) compared to cow milk khoa (2.00%) and buffalo milk khoa (1.05%). The reason could be the use of microbiologically low quality, metalic contaminated, and/or adulterated milk for the manufacture of commercial khoa.

The nine identified carbonyls in the three types of samples (Fig.) show that it was possible to identify more than 50% of the carbonyls in the fresh market samples, while the minimum percentage of carbonyls was identified in the fresh samples of khoa from buffalo milk. In other words, the proportions of unidentified carbonyls remained minimum in the market samples, maximum with samples prepared from buffalo's milk and intermediary in cow milk khoa. The differences in chromatograms (Fig.) for the 3 types of khoa may be mainly attributed to different types of milk used for preparing corresponding samples of khoa under the two conditions.

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CORROSION OF TINPLATE WITH PINEAPPLE JUICE

The nature of different fractions of pineapple juice and their influence on the corrosion of tinplate have been studied. The anionic fraction consisting of organic acids was found responsible for corrosion of tinplate. Tinplate with tin coating of 22.4g/m² was found suitable for canning pineapple products.

In India, large quantities of pineapple are being packed in cans as slices, juice and jam for internal trade and for export. Permissible limits of tin content in the canned pineapple products vary from 100 to 250 ppm. The nature of corrosion of pineapple constituents on tinplate is not known. Mahadeviah *et al*[1,2] have studied the influence of different fractions of mango pulp and orange juice on the corrosion of tinplate. Preliminary experiments were carried out to study the nature of different fractions of pineapple juice and their influence on the corrosion of tinplate. The results are presented in this note. Organic acid, amino acid and sugar farctions in pineapple juice were separated by the method of Hussein *et al*[3]. and detected by paper chromatography[4–6]. Corrosion rate was expressed by determining the percentage loss in weight of the tinplate strips used in the model system[1].

Pineapple juice contained 0.8 per cent acidity (as citric acid), 13° Brix (total soluble solids), 11 per cent reducing sugar and had 3.8 pH. The organic acids comprised citric acid and malic acid, (citric acid is more predominant than malic acid).

Sugars detected were sucrose, glucose and fructose and the amino acids were leucine, methionine, threonine, glutamic acid, aspartic acid, arginine, lysine, and cystine.

Corrosion studies in model system: Tin plate strips (electrolytic-1 lb/base box (22.4 g/m²) were cut into strips of 8 cm × 2 cm with the edges coated with lacquer placed in test tubes covered with different fractions of pineapple juice (different fractions were maintained to the original pH of the pineapple juice) preheated to 85°C, and the tubes were exhausted in boiling water for 10 min to expel oxygen and then sealed. They were stored at 37°C and the corrosion rate of tinplate strips was determined periodically.

As shown in Fig. 1, the anicnic fraction consisting of organic acids was responsible for corrosion of tinplate strips. Organic acids accelerated corrosion in the presence of sugar also. After 12 months' of storage, heavy feathering with only slight detinning was noticed in both these cases. This indicates that the pineapple products can be safely packed in tinplate with tin coating weight of 1 lb/base box (22.4 g/m²). In the presence of sugar and amino acids only light feathering without detinning

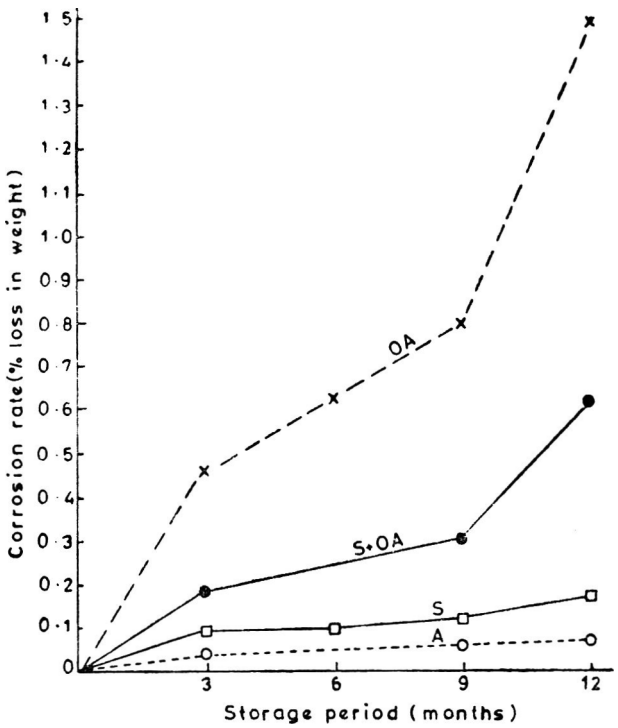

Fig. 1. Influence of different fractions of pineapple juice on corrosion.

A, Amino acid fraction; S, Sugar fraction;
S+OA, Sugar+Organic acid fraction; and
OA, Organic acid fraction.

was observed and corrosion rate was negligible. Similar results were obtained with different fractions of mango pulp and orange juice[1,2].

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EFFECT OF STORAGE TEMPERATURES ON THE QUALITY OF PHALSA BEVERAGE

A ready-to-serve beverage from 'Phalsa' fruit was formulated and stored at different temperatures. Colour in the beverage was best retained at 3°C, followed by 20°C and at room temperature (31-36°C). The beverage can be best stored upto 100 days. The acceptability of the beverage goes down when stored at 20°C or at room temperature.

'Phalsa' (*Grewia subinaequalis* L.) belongs to the family Tilliaceae. The fruits possess a very attractive colour, ranging from crimson red to dark purple due to the presence of delphinidin-3-glucoside and cyanidin-3-glucoside[1]. The extracted juice is of deep crimson red colour, besides a pleasing flavour. The juice is extremely refreshing and is considered to have a cooling effect especially in the hot summer[2]. Investigations have been carried out to study the keeping quality of the beverage prepared from the 'Phalsa' juice.

Mature fruits were obtained from the experimental orchard of the Institute. The fruits were washed in running water, drained and crushed in an apple grater. It was heated to 50°C and pressed in a wooden basket press using a filter cloth to get the juice. A ready-to serve(RTS) beverage containing 35% 'Phalsa' juice was prepared and heated to 85°C and filled, hot into clean, sterilised, colourless glass bottles (200 ml), sealed and cooled immediately in the air. The processed beverage was stored at 3°C, 20°C and at room temperature (31-36°C), for over a period of 100 days.

Physico-chemical Characteristics: TSS (°Brix) were determined with a hand refractometer and the values were corrected to 20°C. Sugars were estimated by the Lane and Eynon's method[3]. Acidity, total phenolics, total ash, iron and specific gravity were determined by the standard methods of A.O.A.C[4]. Ascorbic acid was determined by the method of Robinson and Stotz[5]. Calcium was estimated by the method of Chang and Bray[6]. Hydroxymethyl furfural (HMF) was determined by the method of Luh *et al.*[7] Organoleptic evaluation was carried out by a panel of seven judges using 9-point Hedonic scale[8]. The total anthocyanin pigments were measured by the method of Fuleki and Francis[9] with slight modification. The sample was centrifuged at 5000 rpm for 10 min in a refrigerated centrifuge K-70(GDR). To a 0.2 ml of the supernatant, KCl-HCl buffer (pH 1.0) was added to make up the volume to 10 ml. For the maximum development of colour, the solution was kept at room temperature for 2 hr in the dark. This solution had maximum absorption at 510 nm (Spectronic-20). Subsequent measurements were, therefore, taken at this wavelength.

TABLE 1. PHYSICO-CHEMICAL CONSTITUENTS OF 'PHALSA' JUICE

Juice yield (%)	:	67.50
Pomace (%)	:	32.50
Visible colour	:	crimson red
Specific gravity (33/33°C)	:	1.0959
Total soluble solids (°Brix-at 20°C)	:	20.9
Total acidity (% w/v) (as anhydrous citric acid)	:	2.48
pH	:	3.20
Reducing sugars (%)	:	18.12
Total sugars (%)	:	17.98
Total phenolics (mg/100 ml)	:	280
Total anthocyanins (mg/100 ml)	:	218
Total ash (mg/100 ml)	:	1150
Acid insoluble ash (mg/100 ml)	:	370
Iron (mg/100 ml)	:	0.68
Calcium (mg/100ml)	:	30.11

Results are expressed as mg cyanin per 100 ml beverage.

The crushed fruit was heated to 50°C in order to obtain the maximum juice[1] recovery along with other constituents as shown in Table 1. Like fruits, the juice is also a good source of phenolics, anthocyanins, acids, carbohydrates and minerals particularly iron and calcium. No ascorbic acid was observed, as the fruit itself is a poor source of ascorbic acid[10].

The extracted juice cannot be consumed as such due to its high acid content. The juice thus has to be converted into an RTS beverage for a better sugar/acid blend. An RTS beverage with a °Brix/acid ratio of 25.0 was liked by a panel of seven judges as the best. This composition was subsequently used to carry out experiments on storage studies (Table 2).

The anthocyanins in the heat processed beverage were more stable at 3°C than at 20°C or at room temperature. It was also observed that stored beverage should contain a minimum of 10 mg anthocyanins per 100 ml for its overall organoleptic acceptability[1]. On this basis, beverage can be stored for more than 100 days at 3°C and at the lowest level of acceptability upto 100 days at 20°C and for less than 20 days at room temperature (31-36°C).

Not much change in TSS, acidity and total sugar was observed in the beverage during storage at all the three temperatures. The pH, however, remained stable. A gradual increase in reducing sugars content from 8.58 to 11.48% was noticed in the beverage during 20 days' storage at room temperature. This could be explained as due to the continuous hydrolysis of non-reducing (sucrose) to reducing sugars. This rate of increase in reducing sugars was much slower at 20°C and 3°C upto

TABLE 2. CHANGES IN CHEMICAL CONSTITUENTS AND ORGANOLEPTIC QUALITY OF HEAT PROCESSED 'PHALSA' BEVERAGE DURING STORAGE IN GLASS BOTTLES

Storage temp (°C)	'Storage period (days)	Total antho-cyanins (mg/100ml)	TSS (°Brix at 20°C)	Acidity (%)	pH	Sugars		Hydroxy methyl furfural (mg/l.)	Overall organole-ptic Score*
						Reducing (%)	Total (%)		
Initial	0	22.0	20.5	0.83	3.4	8.58	18.84	Nil	8.5
31-36 (RT)	5	17.5	20.7	0.83	3.4	9.49	18.81	Trace	8.0
31-36 (RT)	10	14.5	20.7	0.83	3.4	10.28	18.84	2.6	7.5
32-36 (RT)	20	9.0	20.7	0.79	3.4	11.48	18.84	4.4	5.0
20	100	10.0	21.1	0.79	3.4	10.17	19.77	1.4	6.3
3	100	19.0	20.5	0.83	3.4	9.19	19.47	Nil	7.4

*The beverage was acceptable at 5.5 and above.

100 days storage. A gradual increase in browning and HMF formation was observed in the beverage with increasing storage period. This was more pronounced at room temperature, than at 20°C.

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PREVENTION OF GERMINATION AND SPOILAGE OF SUBMERGED PADDY

Immersion of soaked or submerged panicles of the sheaves in 5 per cent common salt solution prevented sprouting in paddy grain. Further, the grain threshed from such panicles could be stored in gunny bags without spoilage, mould development, discolouration and off-odour for 10-12 days. The method is cheap and can save the grain from such soaked conditions in cyclone prone areas.

Rice crop in monsoon season is confronted with two major problems viz., (*i*) high moisture content of grain due to high humidity while harvesting, and (*ii*) sprouting due to rains at ripening and harvesting stages. Efforts have been made by Shivanna and Kudrathullah[2] and Antoni Raj *et al*[1]. to preserve the high moisture grain by chemical means. They used sodium chloride as such or in powder form for packing paddy which helped in reducing the moisture content and consequently prevented germination and spoilage. The second problem is not recurrent but serious and occurs when cyclone or heavy down-pour takes place just after harvest and when the sheaves are spread in the field for drying. This results in submergence of sheaves for 2-5 days, and grains suffer from germination, discolouration and mould development. In such situations, farmers have been experiencing heavy to complete loss of the produce. In 1979 kharif season, nearly 6 lakh acres of crop was completely damaged in Andhra Pradesh alone due to cyclone which occurred just after harvest of the crop. This paper describes the recent efforts made with different chemicals on their feasibility for preventing germination of such submerged and soaked paddy.

Chemical treatments for inhibiting germination: The chemicals used include calcium chloride, sodium chloride,

copper sulphate, maleic hydrazide and thiram. In the preliminary study the grain of 'Mahsuri' harvested and soaked for 2 and 4 days in water and subsequently immersed in the chemical solutions for 10 min. at the specified concentrations was tested for germination by maintaining in petri dishes. Tap water served as control.

The study was repeated under field conditions with the same chemicals at the respective concentrations. Harvested sheaves were submerged in water for 2 and 4 days in the field simulating the cyclone affected field conditions. The chemical treatment was given by (*i*) sprinkling these chemicals on the panicles of the sheaves, and by (*ii*) just immersing and taking out the panicles (till the running water from the panicles stops).

Further, various combinations of sodium chloride with copper sulphate, potassium chromate and bleaching powder have also been tried by adopting both the methods described above. The harvested sheaves were again submerged for 2 and 4 days and these chemicals were sprinkled on the panicles and as indicated earlier panicles had also been just immersed and taken out. The treated sheaves were stacked in open in concentric rings with the panicles converged to the centre as practised by the farmers. The atmospheric relative humidity was 45 to 50 per cent and the temperature was 30-32°C.

The grains threshed after salt treatment were preserved in different containers like gunny bags, poly-coated gunny bags and polythene bags by taking 50 kg of grains in three replications. Periodic observations were made on germination, mould development, grain discolouration, off-odour and caking of grain. In these studies paddy submerged for 2 and 4 days without any chemical treatment served as control. The paddy preserved in the bags was milled on tenth day after drying in sun and the percentage of brokens were recorded. The study was repeated thrice.

Preliminary studies in petri dishes with calcium chloride, sodium chloride, copper sulphate and maleic hydrazide indicated their effectiveness at higher concentrations in inhibiting germination. In both sprinkling and dipping (immersion) methods, except sodium chloride at 5 per cent, the other treatments showed moderate to high germination and full development of moulds. However, dipping in sodium chloride at 5 per cent level was effective and did not show any germination or mould development even after 12th day of the treatment. In sprinkling, slight germination and mould development were observed probably because of the irregular wetting of the grain. When the chemicals were combined with sodium chloride it was found that dipping was more effective than sprinkling. Potassium chromate, copper sulphate and bleaching powder when used alone were not effective. This clearly shows that sodium chloride with any other chemical was more effective.

The study was repeated by simulating the submerged conditions in the field and allowing the sheaves to soak for 2 and 4 days. Soaked sheaves were immersed in 5 per cent sodium chloride solution and stacked as practised by the farmers. There was no germination for a period of 12 days while in control it germinated within 2-3 days. This clearly shows that common salt was effective in stopping the enzymatic activity temporarily. Further, neither discolouration nor mould development was noticed in the paddy for 10-12 days. Shivanna and Kudrathullah[2] have applied dry salt powder to high moisture paddy for preservation. Their aim was not to prevent germination in the panicles soaked in water. Their approach was use of salt as a prophylactic measure to prevent sprouting under high humid conditions. In the current studies the relative humidity ranged between 30 and 40 per cent and it was essentially a soaking effect.

The method requires 75 kg of salt (costing Rs. 22.50) dissolved in 1500 of water to treat the sheaves of an acre of paddy crop. Moreover, varied degrees of sprouting like initiation of sprouting, slightly emerged plu-

TABLE 1. INFLUENCE OF SALT TREATMENT ON STORAGE IN DIFFERENT CONTAINERS

Container	Sample	Germination	Mould	Discolouration	Off-odour	Others
Gunny bag	Treated	—	—	—	—	—
	Control	+	Stray	+	—	Caking
Poly-coated gunny bag	Treated	Traces	—	—	—	—
	Control	+	+	—	—	Caking, musty smell
Polythene bag	Treated	—	—	+	+	—
	Control	—	—	+	+	—

Sheaves submerged under field conditions for 4 days, treated with 5% salt solution and threshed. Condition at 10 days after treatment.

+ , Present; —, Nil.

TABLE 2. EFFECT OF SALT TREATMENT ON KERNEL BREAKAGE (MAHSURI).

Packing material	Brokens (%)	
	5% NaCl treated	Control
Heaped with panicles upward	30	43
Stacked (irregularly)	34	Germinated
Gunny bag	34	,,
Poly-coated gunny bag	50	,,
Polythene bag	48	62
Control (no soaking)	26	—

Sheaves submerged under field conditions for 4 days, treated, threshed and stored. Condition at 10 days after treatment.

mules or slightly elongated radicles all get completely dried up. In such cases the affected grain could be utilized for flour or for rava or for beaten rice purposes. Thus, this method serves as an effective and potential saving measure and comes to the rescue of the farmers in such critical times.

Immediately after cyclone, inclement weather is likely to prolong affecting threshing and drying operations. Therefore, storage quality of the treated paddy was studied by storing the grain threshed after salt treatment in gunny bags, poly-coated gunny bags and polythene bags. The observations made 12 days after the treatment (Table 1) clearly indicate that the method is effective in all the containers. Germination was inhibited in polythene bags even when control samples were stored. This could be mainly due to the lack of gaseous exchange. However, such control samples showed mould development, discolouration and off-odour. Thus, the treated paddy could be safely preserved in normal gunny bags for 10 days without germination, mould development, discolouration and off-odour. This helps in gaining time for further drying as per feasibilities. Earlier workers[1] have used dry salt powder by coating to some inert materials like husk. After the completion of the prescribed storage period such materials are to be separated by sieving or eliminated by some other means. But in the current method, there are no such problems as grain had hardly any coating of the salt.

The percentage of brokens in such grain (Table 2) varied from 30 to 35 per cent while in the control all the grains have germinated. Interestingly, in the case of polythene bag containers, the broken percentage was 62 (highest recorded), while it was 48 with salt treatment. Thus, the data indicate that salt treatment also minimises the breakage of rice.

The authors are grateful to Dr. R. Seetharaman, Project Director, All India Coordinated Rice Improvement Project, Hyderabad for his encouragement in carrying out these studies.

All India Cordinated Ricc Improvement Project, Rajendranagar, Hyderabad-500 030.

B. VENKATESWARLU
V. SOMASUNDARA RAO

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PACKAGING SYSTEMS FOR PROTECTING HIGH MOISTURE PADDY

Air tight storage in tins and under soil with suitable chemical treatment preserved high moisture paddy free from spoilage in humid weather. An attempt was made to select composite liners available commercially to act as an effective air barrier. The combination of gunny + PVC (Polivinyl chloride) 150 g + HDPE (High Density Polyethylene) 300 g was better in preventing air entry while the composite bags allowed air entry. It would be better if fabrication of composite bags having desired qualities of gunny/PVC/HDPE combination is explored.

During continuous and incessant downpour, spoilage sets in paddy with high moisture. The harvest of *kuruvai* paddy crop in Tamil Nadu (India) coincides with heavy downpour and consequently the grain moisture at harvest is high leading to spoilage due to sprouting and mould growth. A dry matter loss of 12% was observed when 23% moisture paddy was stored for about 10 days during inclement weather[1]. Since sundrying is not possible under such weather and mechanical drying is costlier, a chemical treatment followed by a packaging technique was developed for preserving moist grains for a short period[2]. This technique requires an air-tight container sac to prevent free air exchange between the grain mass and external atmosphere and hence various liners and composite sacs available in the market were tested and the results are reported here.

Freshly harvested 'ADT 31' *kuruvai* paddy or artificially moistened 'IR 20' paddy with a moisture content of 25 per cent was used. Whenever paddy with high moisture was stored under air tight condition there was development of fermentative odour. This was

TABLE 1. EFFECT OF DIFFERENT PACKAGING SYSTEMS ON PRESERVATION OF MOIST PADDY

Particulars of packages	Germination	Fungal growth	Caking	Air entry
B. Twill gunny + HDPE (300 *g*) loose liner inside	—	+	—	> 3
B. Twill gunny + PVC (150 *g*) loose liner inside	+	+ + +	+	> 1
B. Twill gunny + HDPE (300 *g*) inside + PVC 150 g outside	—	—	—	Curtailed
Bitumen lined gunny bag	+ + +	+ + +	+	- 2
Bitumen lined gunny bag + LDPE (150 *g*) loose liner	+ +	+ +	+	< 4
Bitumen lined gunny bag + PVC (150 *g*) outside + LDPE (150 *g*) inside	+	+	+	< 5
Laminated woven sac with no loose liner	—	+ +	—	> 5
Laminated woven sac with HDPE 300 *g* loose liner	—	+	—	< 7
Sandwiched-Polytape Sandwiched bag	—	+	—	< 8
Sandwiched bag + PVC 150 *g* loose liner	—	+	—	< 9
Sandwiched bag + LDPE 150 *g* loose liner	—	+	—	< 10
Sandwiched bag + HDPE 300 *g* loose liner	—	+	—	> 8 to 11
Polytape laminated	+	+	—	> 12
Polytape grass bag	+	+	—	> all others
Polycel bags	+	+	—	> 3
Rubber bag	+	+	—	> 3
Butyl rubber bag	+	+	—	> 3
Rexin bag	+	+	—	> 3
Nylon rexin	+	+	—	> 3
Gunny - tar coated	+	+	—	- 4
Fertilizer gunny	+ +	+ +	—	- 19
Hessian -gunny	+	+	—	> 19 & 20
3-ply laminated	+	+	—	< 19 to 21
7-ply laminated	+	+	—	> 3

+ ..... + + + : In the order of increasing intensity —: Absence.
The material was examined for 6 days.

prevented by the application of a mixture of camphor (5g), Citronella (0.05 ml), chloroxylenol (0.05 ml) and made up to 100 ml with carbon tetrachloride. Moist grains were treated with 0.1 % (v/w) of the mixture and packed in the following packages. Although the liners varied in their filling capacity, for the sake of uniformity, 40 kg of treated paddy was filled in each sac and tied or stitched. The liners like low density polyethylene (LDPE), high density polyethylene (HDPE), 150 gauge polyvinyl chloride (PVC), polytape laminated with 100 gauge LDPE, polytape grass bag, polytape plain, sandwiched bags (polytape on both the sides with a layer of polyethylene in-between), polycel bags (one layer of cellophane and one layer of polyethylene fastened into a single layer), rubber bag (nylon based-rubber vulcanised), cotton based rexin, nylon based rexin, ordinary cotton bags, tar coated gunny sacs, fertilizer gunny, hessian-gunny, 3-ply laminated gunny, 7-ply laminated gunny and gunny with 150 gauge PVC and 300 gauge HDPE as loose liners were tried. The moist grains were preserved by the chemical applied when the air exchange was totally curtailed. However they showed traces of fungal growth under restricted air diffusion and profuse germination when there is free air exchange. This phenomenon was taken as the test for determining the extent of air entry through the sacs. The experiments with chemical treatments revealed that a condition of restricted aerobic or nearly air-tight condition reduced the oxygen tension and preserved the grains safe. It has been observed that 300 gauge HDPE, was satisfactory even though it did not render full protection to the moist grains (Table 1). Of the combinations tried only gunny, PVC 150 gauge, HDPE 300 gauge combination was found to keep the moist grains free from spoilage.

When the liners were used individually, they did not stand the weight of the paddy mass held in the gunny, the liners were not air-tight as the air entry had occurred through the seams and also through the body of the sacs. With thicker gauge bags like HDPE 1000 gauge, 800 gauge, seven ply laminated gunny bags, etc tying was the problem. In all the loose liners and polytape

bags, handling was difficult as the use of hooks pierced the bags making way for air entry and stacking them one over the other was difficult as the surface of the bags were smooth and slippery. The stitching on the sides and bottom of the sacs provided holes permitting free air entry. This was evident from profuse germination of grains along the stitch lines and negligible germination in grains covered by the body of the sac. In tubular sacs the stitch lines were only at the bottom of the sacs and consequently the damge was severe on this side only. Further in the case of combinations of loose liners placing one inside the other and tying their mouth securely to create airtight condition was difficult. With the above background fabrication of a cheap and durable composite bag incorporating all the characteristics of the loose liners like gunny-PVC (150 gauge) + 300 gauge HDPE. is needed. Such bags may also be useful to transport moist and drenched paddy to other places for immediate processing so as to avoid damage.

Sincere thanks are due to late Dr. V. Subrahmanyan and Shri P. Pillaiyar, Deputy Manager (Tech.) for their guidance.

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BOOK REVIEWS

Antimicrobial Food Additives—Characteristics, Uses, Effects: by Leuck, translated from German by Grant F. Edwards, Springer-Verlag GmbH & Co. KG. Heidelberger, Platz 3 Postfach D-1000, Berlin 33, 1980, pp 280, Price: $39.00.

This is a very useful compilation of valuable information on preservation of foods by chemical means. It gives an excellent review of all aspects of food preservation by chemical techniques; the majority of which involves use of chemical additives. The book has two main sections, i.e., (1) general section dealing with the importance of all preservatives, (*ii*) special chapters on the properties and use of individual preservatives. The sequence of chapters of the book follows the usual system employed in inorganic and organic chemistry. It deals firstly with the aim and development of food preservation followed by analytical detection of preservatives, both qualitative and quantitative, health aspects, legal situation relevant to food, anti-microbial action of preservatives followed by separate chapters devoted to individual preservatives like sodium chloride, silver, boric acid, carbon dioxide, nitrogen, nitrates, nitrites, ozone, hydrogen peroxide, sulphur dioxide, chlorine, ethyl alcohol, ethylene oxide, sucrose, hexamethylenetetramine, formic acid, acetic acid, esters, benzoic acid, salicylic acid, esters of p-hydroxy-thiabendazole, nisin, pimaricin and other preservatives followed by a chapter on packaging and coating. Preservatives which are of minor importance are summarised under 'other preservatives'. Mostly the book confines strictly to food preservation and only a brief reference is made to food analysis.

This book will be useful for a practical man in the food industry with an interest in the scientific aspects of his work. The scientific principles of food preservation are particularly explained in sufficient details for clear understanding in the manner they are used. In the chapters dealing with individual preservatives, a clear attempt has been made to provide systematic description enabling the student or reader to obtain rapid overall picture. A book of this type can also serve as a source of information for Government authorities, medical practitioners, nutritional scientists and not the least educated layman. In the context of the present day controversy regarding restrictions, on the use of food additives, this book will help bring objectivity to all emotionally charged discussions on the use of preservatives in foods to such discussions to a scientific level.

O. P. KAPUR
C.F.T.R.I., MYSORE.

Natural Sulfur Compounds: Novel Biochemical and structural aspects: Ed. by Doriano Cavallini, Gerald E. Gauli and Vincenzo Zappia, Plenum Press, N. Y. and London, 1980.

This volume has 47 research papers presented at the Third International Symposium on "Low Molecular Weight Sulfur Containing Natural Products" sponsored by International Union of Pure and Applied Chemistry and held at Rome (June 1979).

A wide spectrum of disciplines covering organic chemistry, physical chemistry, biology and medicine aspects in the area of sulphur containing compounds is covered.

For the organic and physical chemists, papers on semisynthetic cephalosporins, chemistry of sulphur containing nuphar alkaloids, sulphur compounds in mustelids and synthetic suplhur analogues such as thialysine and thiaproline are of immediate interest.

The biosynthesis of homocysteine in plants and the biological activity of 1,2 dithiolane derivative from mangrove plants are discussed and provide particularly useful information for plant biochemists. Contributions in biochemical and enzymological aspects cover a wide area in respect of transmethylases: the stereochemical aspects and mode of catalysis; involvement of adenosyl methionine in nucleic acid modification; protein methylation; and phospholipid metabolism in brain are some of the interesting facets discussed.

Studies on inhibition of synthesis of glutathione and glutamine by certain methionine analogues have been succintly reviewed by A. Meister.

The current interest on the possible physiological significance and function of taurine is amply reflected in this volume. Over 16 contributions discuss taurine metabolism and in summary provide a good review of the biochemistry and physiological significance of taurine. Taurine apparently is implicated functionally in a diverse number of biological functions: as a osmoregulator in invertebrates; in bile acid conjugation; membrane stabilizing function and as a neurotransmitter. In taurine deficiency, retinal degeneration in cats has been reported. One reviewer in this volume suggests that taurine deficiency is hence of concern in human nutrition.

This collection of papers on sulphur containing compounds is certainly multidisciplinary in nature and provides for research groups interested in chemical and biological aspects of such compounds, an updated review particularly on enzymological and metabolic aspects

of transmethylates, taurine formation and its functional role(s).

P. B. RAMA RAO
C. F. T. R. I., MYSORE.

Proceedings of the International Scientific Colloquium on Coffee: held on Nov. 26-Dec. 3, 1977, Association Scientific International du Cafe 34, rue des Renaudes-57017, Paris.

Once again the ASIC has brought out detailed proceedings of the colloquium held in Abidjan on Coffee in a significant way. The papers presented are covered under the heading: analysis and chemical composition, chemical studies related to technology, physiological studies, agronomy and other global subjects. The ASIC has once again provided a platform to discuss the present state of knowledge about coffee which is a major stimulant beverage. Raw product quality, technology, effects of coffee under physiological and pathological conditions and composition of bean and its changes have been the main coverage of papers. Dr. Smith has also given a review of the recent developments with an extensive bibliography.

There has been an appreciable interest in coffee research since the 7th Conference. Nearly 200 participants from various countries like Germany, Angola, Belgium, Brazil, France, Ghana, U. K., Italy, Kenya, Nigeria, Switzerland, Tanzania and Tokyo, attended the conference. The representation from Ivory Coast was the largest. India was singularly absent eventhough considerable work is in progress on coffee in India.

Analysis: In the section on analysis the following studies are significant. Chemical composition and variations, biosynthesis and metabolism of coffee especially the formation of phenolic acid trigonelline and terpenoids. High pressure liquid chromatography has been used for the determination of phenolic acid. Liquid chromatographic studies of coffee wax, tracer studies on biogenesis of caffeine, caffeine production in tissue culture studies and headspace techniques for aroma profiles are important studies.

In chemistry and technological studies, packaging and marketing of roasted coffee, use of mathematical models to aid coffee transaction, biochemical and organoleptic changes in raw coffee, quality deterioration due to hydrolytic oxidative enzymes and microorganisms and subsequent changes in lipid and phenolic distribution patterns.

Technology: In technology survey of different processing options in soluble coffee manufacture, advantages of pro-concentration of extract before drying, fluidised bed drying and preservation of aroma during drying by different methods are worthy of consideration.

In sensory evaluation, studies on influence of extraction parameters on the bitter taste of coffee indicate that bitterness increases with an increase in proportion of ground coffee to water, time and temperature. In a study of brewing technique a range of commercially available domestic coffee devices have been studied for performance and sensory quality.

Influence of soaking on the evolution of various compounds capable of diffusing like caffeine and chlorogenic acids have been studied.

Physiology: Molecular and cellular sites of action of caffeine, sensory and central nervous effects of caffeine, pharmocology of 14-C-cinamic acid compounds in rats, relationship of coffee drinking to death and cardiovascular disease, incidence of neoplasma and artery sclerosis in rats fed with instant coffee and coffee in pregnancy are the various studies in physiological aspects. No deteriorative symptoms were reported with coffee.

Agronomy: An exhaustive survey on utilisation of genetic resources of the genus coffee for improvement of cultivated coffee based on African studies stresses the need for safeguarding plant heritage and international cooperation in preservation of wild coffee. Analysis of enzymatic polymorphism in the genus coffee by electrophoresis studies indicated great variability in natural coffee.

Survey of collection of wild coffee in Africa has been carried out to constitute variability reservoir for the purpose of improvement.

Hybridization between *Coffee canephora* and *C. eugeniodes* has been tried for improving the quality of low altitude coffee.

The tetraploid hybrid between *C. canephora* and *C. arabica* called *C. arbusta* and its performance in Ivory Coast has been presented. Technical innovation in coffee culture like propogation by cutting, tissue culture, grafting and bud grafting for intensive planting, scientific fertilisation and harvesting are reviewed. Cuba studies in classification of *C. arabica* and comparative study on rooting media, indicated that rooting media influences rooting percentage, the remarkable one being rice hulls.

Detailed study on irrigation of robusta coffee in Ivory Coast is a significant study.

Other studies are on coffee rust from Kenya, Timor, Ivory Coast, Cameroun and Portugal.

This colloquium is a great success in bringing together workers on different aspects of coffee. The studies have been significant and show immense potential for improvement of coffee. This compilation should be

very useful to all research institutions working on coffee.

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

Advances in Fish Science and Technology: Papers presented at the Jubilee Conference of the Torry Research Station, Aberdeen, Scotland, 23-27 July 1979. Edited by J. J. Connel, Fishing News Books Ltd., Farnham, Surrey, England, pp. 512.

Torry Research Station, Aberdeen, Scotland needs little introduction to fish scientists in particular and food scientists/technologists in general. Fifty years back when it was started, it mainly concerned itself with chilling practises on board fishing vessels. Today it encompasses the entire spectrum of fish research and development including advisory service to fish industry. The book is a compilation of most of the papers presented at an international conference on the occasion of 50th anniversary of Torry research station.

It is broadly divided into two sections; the review section and the recent advances section.

The first chapter, which reviews the past, present and future of fish technology, consists of six papers. These describe the fish resources, the origins and influence of scientific and technical ideas concerning fish as food, methods of fish handling, fish preservation, a statistical analysis of the utilisation and quality control. These papers give a comprehensive review of the progress of fish technology made during the last 50 years, each one is supplemented by extensive references.

The nine reviews of the chapter two entitled past, present and future of fish science, present the progress in the scientific knowledge and ideas regarding fish science. This covers areas such as structure of proteins of shell fish, organic and inorganic components, changes in fish lipids, the nutritional value of fish to humans, microbiology, sensory evaluation and application of engineering science to fish preservation.

The second section, namely, the recent advances consists of 14 chapters. The first chapter embraces all the aspects of the minced fish; the six papers cover raw materials, effect of time, temperature, additives, pretreatment methods on the quality of minced fish and other products like fish patties.

The next chapter describes the processes and new products. Papers presented in this chapter increase significantly the understanding of various aspects of the field. The chemistry of smoking and analytical methods for investigating the changes in smoking are presented in the subsequent chapter. Chapter six covers chilled and frozen storage-a field, the importance of which in fish technology is well recognised.

Krill is not being fully utilised mainly due to economic reasons. Very useful information on properties and nutritional aspects is provided in the chapter on Krill. Utilisation of by-products for economic viability is very important to fish industry. Efficient utilisation, disposal and acceptable solutions to problems are discussed in the chapter on by-products. Technology transfer in fish industry is fully discussed in two papers. Importance of the three species which are not being fully utilised, namely, mackerels, blue whiting and mandi is dealt with in a separate chapter.

As the consumer becomes more and more demanding, methods of quality assessment of a product need continuous updating. Details of sensory and non-sensory methods for monitoring quality are a thoughtful inclusion.

Chapter twelve draws together eight papers on fish proteins. Various protein interactions, cross-linkages and electrophoresis for studying fish proteins are adequately included. Microbiological aspects form the thirteenth chapter. Low molecular weight compounds, the important role of water in fish and histology of blue whiting are discussed in the last three chapters.

This extensive compilation of valuable materials which gives a background information on the current state of fish science and technology will be a very useful source of information to the basic scientists and technologists connected with fishing industry.

T. R. SHARMA
DEFENCE FOOD RESEARCH LABORATORY, MYSORE.

ASSOCIATION NEWS

Hyderabad Chapter

The Chapter has brought out seven issues of its news chronicle *Food for Thought*. The Chapter thanks the Government of Andhra Pradesh for the generous financial support.

Seven lectures were delivered by eminent experts on the following subjects: *sanitation in food proceessing plants* (Dr. S. Dalal); *importance of nutrition education* (Mr. B. D. Tripathi); *Vegetable oil industry-production and distribution* (Dr. K. T. Achaya); *management of inland marine fish production in India* (Prof. Hingorani); *dairy industry in Andhra Pradesh* (Dr. K. Madhava Rao); *the extruded foods and their importance* (Mr. N. Mallikarjuna Rao) and *the recent trends in food science and technology with special reference to R&D programmes of CFTRI, Mysore*, (Mr. M. M. Krishnaiah).

Eight film shows on food processing were screened. A visit to ready-to-eat processed food factory at Nacharam, Hyderabad, was arranged.

The following were elected as the Office Bearers of the Executive Committee for 1981.

President	DR. G. LAKSHMINARAYANA
Vice-President	MRS. YAMUNA RANGA RAO
Secretary	DR. A. J. PANTULU
Treasurer	DR. Y. S. R. SASTRY
Jt. Secretary	MR. SURENDRA KUMAR SOOD

Short Course on Aflatoxin

The Oil Technologists' Association of India, Southern Zone, in collaboration with the Association of Food Scientists and Technologists (India), Hyderabad Chapter and Mycotoxin Research Group, is going to conduct a Short Course on "Aflatoxins in Foods and Feeds" during 2–4 November, 1981 at the National Institute of Nutrition, Hyderabad. The above course mainly deals with the recent developments in the field of fungal spoilage of foods; analytical techniques and detection and quantitation of mycotoxins; and problems concerned with food industries *vis-a-vis* aflatoxins. The number of participants is restricted with due preference to the members of OTAI/AFST/MRG. The participants are expected to be science graduates with chemistry and with 2 years experience in the field. The registration fee is Rs. 400/– for members and Rs. 600/– for non-members. Further particulars can be had from the "Hony. Secretary, OTAI Southern Zone, C/o Regional Research Laboratory, Hyderabad–500 009."

PUBLICATIONS
OF
THE ASSOCIATION OF FOOD SCIENTISTS & TECHNOLOGISTS (I)

(Central Food Technological Research Institute Campus, Mysore)

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1. **REVIEWS IN FOOD SCIENCE AND TECHNOLOGY — VOL. IV**
   Royal 8vo, hard bound, P. 255.
   Price: India **Rs. 8/–**
   Abroad: **$5 Surface mail**
   $8 Air mail.

2. **PROCEEDINGS OF THE SYMPOSIUM ON DEVELOPMENT AND PROSPECTS OF SPICE INDUSTRY IN INDIA**
   Demi–quarto, paper back, P. 100
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   Abroad: **$5 Surface mail**
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3. **PROCEEDINGS OF THE SYMPOSIUM ON FISH PROCESSING INDUSTRY IN INDIA HELD AT CFTRI, MYSORE–1975**
   Demi–quarto, paper back, P. 156
   Price: India **Rs. 10/–**
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REG. NO. 24918/64

JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Vol. 18 No. 5 **Contents of forthcoming issue** **Sept.-Oct. 1981**

Research Papers

Research Notes

Printed and Published by K. R. Sreekantiah, Secretary, AFST (India), CFTRI, Mysore-570013, at Sharada Press, Mangalore-575001

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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