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Volume 18 Number 3 May-June 1981

CONTENTS

Research Papers

Research Notes

RESEARCH PAPERS

Seed Protein Fractions and Amino Acid Composition of Some Wild Species of Pigeon Pea

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The pigeon pea and eight wild related species belonging to the subtribe *Cajaninae* of tribe *Phaseoleae* (Leguminosae) were studied for their seed protein fractions and levels of nonprotein nitrogen. Salt soluble proteins of these species were characterized by SDS—polyacrylamide gel electrophoresis. The electrophoretic pattern revealed similarities in the major protein subunits in these species, although the intensity of these subunits and other minor protein subunits varied. Total seed proteins of wild relatives of pigeon pea were analysed for amino acid composition. Seed protein content of the wild species was higher than that of the cultivated species. Considerable differences in the levels of lysine and sulphur containing amino acids were observed among the wild species.

As in several other grain legumes, the limiting essential amino acids in pigeon pea (*Cajanus cajan*) are the sulphur containing amino acids, methionine and cystine, and tryptophan[1]. The selection of cultivars with higher protein content and better nutritional quality without reduction in yield is one of the objectives of ICRISAT. We have screened several thousand germplasm accessions and have identified a few wild relatives of *Cajanus* having a higher protein content. Earlier attempts to develop high protein lines by crossing the cultivated species with wild relatives has led to some improvement in the protein content of their derivatives[2]. In this paper seed protein fractions, electrophoretic patterns of salt soluble proteins and the amino acid composition of eight wild relatives of pigeon pea are reported.

Materials and Methods

Seed material of wild relatives and cv.'T-21' of pigeon pea grown in the 1976-77 rainy season at ICRISAT Centre, Patancheru, were obtained from the Genetic Resources Unit of ICRISAT. In order to remove the seed coat, samples were soaked in water at 5°C overnight. Excess water was decanted and seed coats were removed manually by use of forceps and samples were dried in the oven at 65°C. Decorticated seed samples were ground in a Udy cyclone mill to pass through a 60-mesh sieve and were defatted using hexane in a Soxhlet apparatus. Nitrogen in the samples was determined by the microKjeldahl procedure[3] and converted into crude protein multiplying with 6.25. Non protein nitrogen (NPN) was extracted using 10 per cent trichloroacetic acid (TCA) as described earlier.[4]

Amino acid analysis: Defatted samples (50 mg) were refluxed in 50 ml of 6N HCl for 24 hr and the acid was removed in a rotary flash evaporator. Residues were taken in a known volume of citrate buffer (pH2.2) and an aliquot of each sample was used for analysis in a Beckman 120-C amino acid analyser.

Extraction of Protein Fractions and Electrophoresis of Salt Soluble Proteins: Defatted flour samples were successively extracted with 0.5 M sodium chloride solution in 0.01 M phosphate buffer (pH 7.0), 0.1 N sodium hydroxide and 70 per cent ethanol to separate the total seed proteins into albumin and globulin fraction, glutelin and prolamin fraction, respectively as described by Osborne[5]. The flour sample (1g) was extracted with the first solvent (15ml), and after centrifugation, the residue was reextracted twice and supernatants were collected and made up to a known volume. Similar steps were carried out with the remaining two solvents. Nitrogen in the protein extracts was determined by the microKjeldahl procedure. Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE) of salt soluble proteins was carried out according to the procedure of Weber and Osborn[6]. For this procedure, protein in the extracts obtained with 0.5 M sodium chloride solution in 0.01 M phosphate buffer (pH 7.0) was determined by the method of Lowry *et al*[7]. The protein solutions were incubated with 0.1 per cent mercaptoethanol and 0.1 per cent SDS solution for 3

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min at 100° C prior to electrophoresis. Extracts of each sample containing equal amount of protein (200 μg) was applied on each gel and all the samples were run concurrently. Human serum albumin (68,000), ovalbumin (45,000) (Worthington Biochemical Corporation. U.S.A.) and myoglobin (17,800) (Sigma Chemical Co., U.S.A) were used as reference proteins.

Results and Discussion

Results of protein solubility fractions of wild relatives and the cultivated species of pigeon pea (cv. 'T-21') are shown in Table 1. The protein content in wild relatives ranged between 28.3 and 30.5, whereas *Cajanus* had 24.2 per cent. Earlier workers[2] have also reported higher protein contents in some wild species compared to the cultivated species. Nonprotein nitrogen ranged between 9.0 and 13.4 per cent among the wild species as compared to the cultivated species which had 11.0 percent (Table 1). Some variation was observed in the levels of salt soluble proteins, albumin and globulin (68.5 to 75.3%) in wild species. The glutelin fraction varied from 15.4 to 20.9 per cent among wild species and was 19.6 per cent in cv. 'T-21'. Again no large differences were observed in the amount of prolamin fractions among the wild species and the cultivated species. This shows that no major differences exist between the wild relatives and cultivated species of pigeon pea as far as the distribution of seed protein fractions is concerned.

SDS-polyacrylamide gel electrophoresis of the salt soluble proteins was carried out to find out if there are any genetic variation in the different subunits of seed storage proteins. The electrophoretic banding patterns of these species are shown in Fig 1. It was observed that two protein subunits with higher molecular weight are

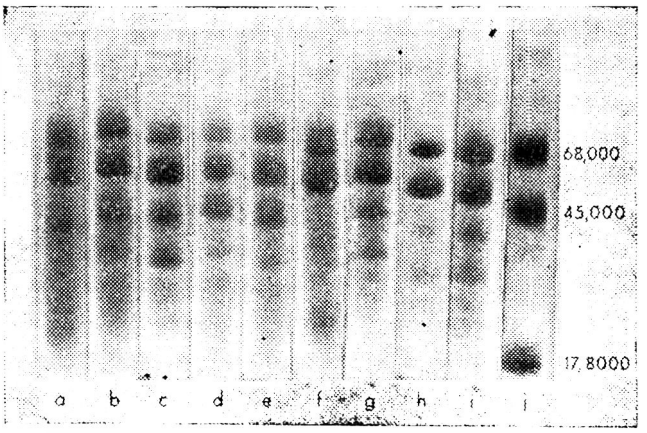

Fig. 1. Electrophoretic patterns of salt soluble proteins of wild relatives and cultivated species: a, *A. albicans;* b, *A. lineata;* c, *A. platycarpa;* d, *A. scarabaeoides;* e, *A. sericea;* f, *A. volubilis;* g, *F. grahamiana;* h, *R. rothii;* i, cv. T-21; j, Reference proteins.

the major components of seed storage proteins and these protein subunits with varying intensity appeared in all the wild relatives including the cultivated species of pigeon pea. However, from the intensity of the bands, it appeared that these protein subunits were present in higher proportion in cv. 'T-21' than in other species. Also, some other differences in the intensity of fast moving protein bands were found. In the case of *Atylosia volubilis* (Fig. 1-f) some of the fast moving bands appeared to be absent when compared with other species. In general, the electrophoretic pattern of salt soluble proteins revealed similarities between the wild relatives and cultivated species and this might perhaps be considered a clue that these species are related to each other with respect to the characteristics of some of

TABLE 1. SEED PROTEIN FRACTIONS (PER CENT OF TOTAL N) OF WILD RELATIVES AND CULTIVATED SPECIES OF PIGEON PEA

Species	Crude protein* (%)	Non protein nitrogen	Albumin + globulin	glutelin	Prolamin	Residue	Total
Atylosia albicans	30.5	10.5	75.3	17.0	3.5	2.2	98.0
A. lineata	29.1	9.7	70.9	19.2	4.2	3.5	97.8
A. platycarpa	29.2	11.3	68.6	20.9	4.8	4.6	98.9
A. scarabaeoides	28.4	12.6	74.2	15.8	3.6	3.0	96.6
A. sericea	29.4	9.0	72.2	16.0	3.8	5.4	97.4
A. volubilis	28.3	13.4	72.9	17.5	3.9	3.3	97.6
Flemingia grahamiana	29.3	10.8	68.5	18.3	5.3	4.7	96.8
Rhynchosia rothii	28.7	9.4	71.8	15.4	2.8	4.1	94.1
Range	28.3 to 30.5	9.0 to 13.4	68.5 to 75.3	15.4 to 20.9	2.8 to 5.3	2.2 to 5.4	
Cajanus cajan (cv. T-21)	24.2	11.0	70.4	19.6	3.1	3.8	96.9

*Moisture free (N×6.25)

TABLE 2. AMINO ACID (G/16G N) COMPOSITION OF WILD RELATIVES AND CULTIVATED SPECIES OF THE PIGEON PEA

Amino acids	*A.albicans*	*A.lineata*	*A.platy-carpa*	*A.scara-baeoides*	*A.sericea*	*A.volubilis*	*Flemingia grahamiana*	*Rhynchosia rothii*	Range	*Cajanus cajan*
Lysine	7.10	6.33	6.95	6.17	6.74	7.48	6.31	6.82	6.17— 7.48	7.06
Histidine	3.27	4.19	3.19	3.44	4.03	3.18	3.61	3.62	3.18— 4.19	4.21
Arginine	5.98	6.54	8.32	8.07	7.55	6.55	6.21	7.72	5.98— 8.32	7.89
Aspartic acid	10.64	10.72	10.75	10.78	10.24	8.82	10.09	10.96	8.82—10.96	10.74
Threonine	3.46	3.84	4.83	4.29	4.32	3.42	3.66	4.19	3.42— 4.83	4.24
Serine	4.83	4.78	5.09	5.73	4.90	4.82	5.06	5.31	4.78— 5.73	6.30
Glutamic acid	25.08	23.75	24.06	23.84	24.19	23.42	22.75	18.93	18.93—25.08	24.71
Proline	4.25	4.20	5.53	4.76	5.11	3.98	4.26	5.10	3.98— 5.53	3.90
Glycine	3.53	4.65	4.70	4.79	4.58	3.79	5.84	4.48	3.53— 5.84	4.57
Alanine	3.24	4.96	5.38	5.27	5.17	4.63	5.72	4.21	3.24— 5.72	5.02
Cystine	0.97	0.90	1.17	1.31	1.15	0.88	1.16	1.58	0.88— 1.58	1.03
Valine	4.71	4.80	6.32	5.18	4.96	4.47	4.84	5.71	4.47— 6.32	5.70
Methionine	1.16	1.17	1.08	1.17	1.34	0.96	1.86	0.75	0.75— 1.86	1.82
Isoleucine	3.66	4.06	4.02	4.40	4.22	4.01	4.23	4.40	3.66— 4.39	4.06
Leucine	8.31	8.95	8.73	9.60	7.88	8.56	8.76	8.39	7.88— 9.60	8.70
Tyrosine	2.75	3.03	3.24	3.27	3.16	2.65	2.75	3.28	2.65— 3.28	3.18
Phenylalanine	10.02	11.00	10.43	9.26	11.31	13.44	12.19	8.20	8.20—13.44	10.01
N. recovery (%)	87.23	89.51	96.13	94.38	92.46	86.46	90.10	88.00	86.46—96.13	91.53

the important genetic constituents. Further studies of specific isoenzyme and cytological tests are required to ascertain the genetic relationship between the cultivated and wild species.

The amino acid composition of wild relatives and cultivated species are shown in Table 2. Tryptophan was not analysed and the reported values for methionine and cystine are probably low as they are partly destroyed during hydrolysis. Also the values reported here are the results of a single determination on unreplicated material, and therefore, the data should be used with caution. Some differences were observed in the levels of essential and non essential amino acids of total seed proteins among the wild relatives. Lysine and phenylalanine levels were higher in *Atylosia volubilis* than in other wild relatives and *Cajanas cajan*, whereas the reserve trend was true for aspartic acid, threonine, cystine and tyrosine. *Atylosia sericea* contained the highest amount of sulphur amino acids, methionine and cystine which showed an appreciable variation among the species. *Atylosia scarabaeoides* had the lowest lysine content of all species. From the nutrition point of view, the levels of both methionine and cystine are important and should be considered together[8]. Moreover, lysine and sulphur amino acids were considered as two of the important protein components in a cereal and legume based diet as they tend to complement each other. Therefore, information regarding the levels of these amino acids in the wild relatives would be of interest to breeders and other concerned scientists in programs that are involved in upgrading the nutritional quality of the grain.

Acknowledgement

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References

1. Bressani, R., *Proc. Symp. Nutritional Improvement of Food Legumes*, PAG of U.N., 1972, 15.
2. Reddy, L. J., Green, J. M., Singh, U., Bisen, S. S. and Jambunathan R., in *Proc. Symp. Seed Protein Improvement in Cereal and Grain Legumes*. FAO/IAEA, 1978, 105.
3. *Official Methods of Analysis, Association of Official Analytical Chemists*, 11th ed, Washington DC, 1970.
4. Singh, U. and Jambunathan R., *J. Agric. Fd Chem.* (in press).
5. Osborne, T. B., *The Vegetable Proteins*, Longman's Green, New York, 1924.
6. Weber, K. and Osborn, M., *J. biol. Chem.*, 1964, **244**, 4406.
7. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. L., *J. biol. Chem.*, 1951, **193**, 265.
8. Boulter, D., Evans, I. M. and Thompson, A., *Qualitas Plantarum, Plant Fd Human Nutr.*, 1976, **26**, 107.

Studies on *Desi* and *Kabuli* Chick-Pea *(Cicer arietinum L.)* Cultivars II. Seed Protein Fractions and Amino Acid Composition

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Amino acid composition of total seed proteins of four cultivars each of *Desi* and *Kabuli* chick-pea was determined. The distribution of various classes of proteins in these cultivars was studied using three different solvents and the salt soluble proteins were characterized by sodium dodecyl sulphate (SDS)—polyacrylamide gel electrophoresis. Very little difference was found between *Kabuli* and *Desi* type chick-pea cultivars and it was not possible to distinguish these cultivars by the techniques employed.

Proteins, in addition to nucleic acids, are species-specific macromolecules and can be used for taxonomic differentiation. Although most of the proteins in legume seeds consist mainly of albumins and globulins and are thus relatively less heterogenous than the cereal proteins, different legume species contain varying amounts and types of protein based on their solubility criteria[1]. Electrophoretic pattern of globulins extracted from different species of legume is known[2].

Within the cultiaved species of chick-pea (*Cicer arietinum L.*)*Desi* and *Kabuli* types are two distinct groups of practical importance. *Desi* types with yellow to brown testa are mostly grown as a "cool season" crop in the tropics. *Kabuli* types with cream coloured testa are generally grown as a summer crop in more temperate zones. *Desi* types account for over 80% of the world's chick-pea production. Earlier workers[3] studied the distribution of nutrients in *Kabuli* and *Desi* cultivars of chick-pea and reported that *Kabuli* cultivars had higher protein, ether extracts, and iron content. But results from our laboratory[4] indicated no significant differences in the levels of protein content, soluble sugars, starch, ether extract, and ash between *Desi* and *Kabuli* types. However, we observed that seed coat percentage and fiber content differ considerably and thus could be used to distinguish between *Desi* and *Kabuli* chick-pea cultivars. This paper describes the results of the study on seed protein fractions, electrophoretic patterns, and amino acid compostion of some *Desi* and *Kabuli* cultivars.

Materials and Methods

Four *Desi* cultivars '(BG-203,', 'Annigeri', 'CPS-1', 'T-3') and four *Kabuli* cultivars ('Rabat', 'Giza', 'L-550', 'K-4') grown at ICRISAT experimental farm, Hissar (29°N), during the post-rainy (rabi) season of 1977-1978 were used in this experiment. Seeds were obtained by pooling seeds from single plots from our chick-pea breeding sections. Dhal (decorticated split seeds) samples were prepared by soaking whole seeds in an excess of distilled water and keeping them at 5°C overnight. After decanting the excess water, seed coats were removed by forceps manually and samples were dried in the oven at 65°C. Dhal samples were ground in a Udy cyclone mill to pass through a 60-mesh sieve and were defatted using n-hexane in a Soxhlet apparatus.

Total nitrogen and non protein nitrogen: Total nitrogen in the samples and extracts was determined by the microKjeldahl method[5] and crude protein content was calculated using a factor of 6.25. Non protein nitrogen (NPN) was extracted using 10% trichloroacetic acid (TCA) as described earlier[6], and nitrogen in the extracts was determined by the microKjeldahl method.

Separation of protein fractions: The defatted flour samples were successively extracted with 0.5 M sodium chloride in 0.01 M phosphate buffer (pH 7.0) solution, 0.1 N sodium hydroxide and 70% ethanol to separate the proteins into albumin and globulin, glutelin and prolamin fractions, respectively, as described earlier[7]. The flour sample (1g) was extracted with the first solvent (15 ml) by shaking in a centriguge tube for 1 hr and centrifuged at 12,000×*g* for 15 min. Supernatant was

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saved and the residue was again extracted twice for 30 min successively with the same solvent and similar steps were carried out using other solvents. Extracts and washings from each step were pooled, made up to a known volume and nitrogen in the extracts was determined by the microKjeldahl procedure.

Extraction and electrophoresis of salt soluble proteins: Defatted flour samples were extracted with 0.5 M sodium chloride in 0.01 M phosphate buffer (pH 7.0) for 1 hr as described above. Protein content in the extracts was estimated as per the procedure of Lowry *et al*[8]. Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis was performed according to the procedure of Weber and Osborn[9] with minor modifications. In the procedure used, protein extracts of samples were treated with 0.1% SDS and 0.1% 2-mercapto-ethanol in 0.01 M phosphate buffer (pH 7.1) for 3 min at 100°C. Aliquots of each sample containing about 200 μg protein were applied on each of the acrylamide gel (7½%) and electrophoresis of all samples was carried out concurrently in 0.1 M phosphate buffer (pH 7.1). Human serum albumin (68,000), ovalbumin (45,000) and myoglobin (17,800) were used as standard reference proteins. Gels were stained in 0.25% Coomassie Brilliant Blue R-250 in a solvent consisting of methanol: water:acetic acid (5:4:1, v/v). Destaining of the gel was accomplished with the same solvent prepared in the ratio of 5:88:7.

Amino acid analysis: Defatted flour samples were hydrolysed by refluxing in 6 N HCl for 24 hr and excess acid was removed using a flash evaporator. Residues were dissolved in citrate buffer (pH 2.2). The amino acids were analysed in an amino acid analyser (Beckman 120C Model).

Results and discussion

The results of the protein fraction data of *Desi* and *Kabuli* samples are shown in Table 1. Protein content ranged between 23.7 and 26.8 for *Desi* cultivars and between 22.0 and 27.0 for *Kabuli* cultivars. The amount of non-protein nitrogen (NPN) was slightly higher in *Kabuli* cultivars (12.3%) as compared to *Desi* cultivars (11.1%). Only small differences in the distribution of seed protein fractions of *Desi* and *Kabuli* types were observed. Among *Desi* cultivars, salt soluble proteins, albumin and globulin varied from 71.9 to 76.8% while in *Kabuli* culivars the values ranged between 75.4% and 80.8. The distribution pattern of glutelin and prolamin fractions did not differ much and hence it was not possible to distinguish between *Desi* and *Kabuli* types based on this criterion.

Electrophoretic patterns of salt soluble proteins of *Desi* and *Kabuli* cultivars revealed the presence of several sub-units (Fig. 1). One major protein component with lower molecular weight was found to be present in all the cultivars studied and the general electrophoretic pattern did not reveal any major differences between the *Desi* and *Kabuli* cultivars. Using electrophoretic technique it has been reported that relative proportions of different storage proteins vary considerably in different species of legumes[2]. Earlier workers also reported that globulin fractions of the different varieties of chick-pea behaved similarly on gel chromatography and sedimentation analysis but there were differences in banding patterns obtained by acrylamide gel electrophoresis[10]. The present investigation failed to reveal any significant difference in the electrophoretic patterns of *Desi* and *Kabuli* cultivars, thus indicating that more specific studies like isoenzymes and their electrophoretic

TABLE 1. SEED PROTEIN FRACTIONS (PER CENT OF TOTAL NITROGEN) OF *DESI* AND *KABULI* CULTIVARS OF CHICK-PEA

Cultivar	Protein* (%)	NPN	Albumin+ globulin	Glutelin	Prolamin	Residue	Total
Desi							
BG-203	26.5	10.7	74.5	20.4	1.8	1.3	98.1
Annegiri	24.2	12.3	76.6	19.8	3.0	1.0	100.4
CPS-1	26.8	12.0	76.8	21.8	1.2	1.4	100.3
T-3	23.7	9.5	71.9	20.6	2.5	1.3	96.3
Range	23.7 to 26.8	9.5 to 12.3	71.9 to 76.8	19.8 to 21.8	1.2 to 3.0	1.0 to 1.4	96.3 to 100.4
Kabuli							
Rabat	24.1	12.6	78.6	18.4	1.4	1.3	99.7
Giza	27.0	13.1	80.8	18.0	1.9	0.9	101.5
L-550	22.2	12.5	79.5	18.9	1.4	0.8	100.5
K-4	24.6	10.9	75.4	19.4	1.8	1.1	97.6
Range	22.2 to 27.0	10.9 to 13.1	75.4 to 80.8	18.0 to 19.4	1.4 to 1.9	0.8 to 1.3	97.6 to 101.5

*Moisture-free (N × 6.25)

TABLE 2. AMINO ACID COMPOSITION (G/16 G N) OF *DESI* AND *KABULI* CULTIVARS OF CHICK-PEA

Amino Acid	*Desi*					*Kabuli*				
	BG-203	Annegiri	T-3	CPS-1	Range	Rabat	Giza	L-550	K-4	Range
Lysine	6.24	6.61	6.61	6.03	6.03- 6.61	6.34	6.82	7.26	6.58	6.34- 7.26
Histidine	2.34	2.31	2.37	2.33	2.31- 2.37	2.12	2.37	2.44	2.26	2.12- 2.44
Arginine	9.97	11.83	9.90	12.49	9.90-12.49	11.03	12.04	9.49	10.44	9.49-12.04
Aspartic acid	11.43	11.42	13.06	11.93	11.42-13.06	10.99	14.15	13.31	12.58	10.99-14.15
Threonine	3.00	3.53	3.48	3.33	3.00- 3.53	3.20	3.46	3.65	3.49	3.20- 3.65
Serine	4.79	4.89	5.15	4.99	4.79- 5.15	4.68	5.39	5.40	5.31	4.68- 5.40
Glutamic acid	19.27	20.20	19.61	18.59	18.59-20.20	19.28	20.36	21.00	20.35	19.29-21.00
Proline	3.60	3.96	4.07	3.78	3.60- 4.07	3.94	4.07	4.34	4.04	3.94- 4.34
Glycine	3.99	4.28	4.08	3.98	3.98- 4.28	3.78	4.13	4.33	4.21	3.78- 4.33
Alanine	4.17	4.52	4.38	4.21	4.17- 4.52	4.03	4.48	4.72	4.55	4.03- 4.72
Cystine	1.11	1.04	1.34	1.35	1.04- 1.35	1.01	1.31	1.48	1.14	1.01- 1.48
Valine	4.13	4.64	4.22	4.04	4.04- 4.64	4.07	4.05	4.36	4.12	4.05- 4.36
Methionine	1.28	1.34	1.24	1.27	1.24- 1.34	1.18	1.32	1.32	1.42	1.18- 1.42
Isoleucine	3.95	4.03	4.03	3.65	3.65- 4.03	3.50	3.90	4.06	3.80	3.50- 4.06
Leucine	7.52	7.91	7.91	7.36	7.36- 7.91	7.05	7.69	8.24	7.75	7.05- 8.24
Tyrosine	2.85	3.00	3.07	2.91	2.91- 3.07	2.70	3.06	3.28	2.98	2.70- 3.28
Phenylalanine	5.32	5.37	5.56	5.22	5.22- 5.56	4.98	5.55	5.90	5.45	4.98- 5.90
N. recovery (%)	85.45	91.05	88.20	88.75	88.36	85.05	95.50	90.97	87.20	

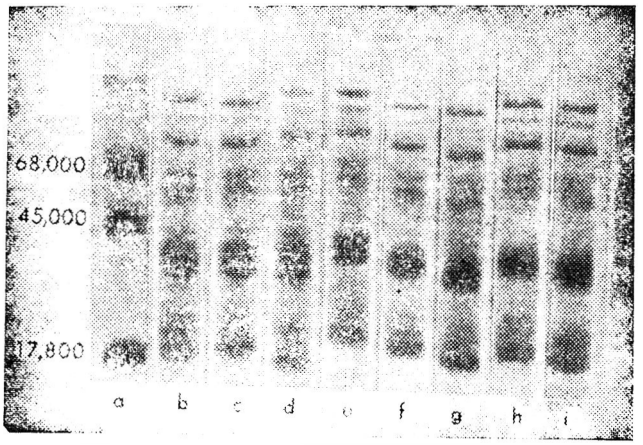

Fig. 1. Electrophoretic patterns of salt soluble proteins of chickpea cultivars a, Standard proteins; b, BG-203; c, Annigeri; d, T-3; e, CPS-1; f, Rabat; g, Giza; h, L-550; i, K-4.

pattern should be undertaken before any conclusions are drawn.

Amino acid composition of *Desi* and *Kabuli* cultivars of chick-pea is shown in Table 2. Lysine content (g/16 g N) ranged between 6.34 and 7.26 for *Kabuli* cultivars, whereas it varied from 6.03 to 6.61 for *Desi* cultivars. Among all the cultivars 'L-550' had the highest lysine (7.26 g/16g N) and sulphur-containing amino acids, methionine and cystine (2.80 g/16g N). Although some variation in the concentration of sulphur-containing amino acids was observed among these cultivars, no noticeable differences were found between *Desi* and *Kabuli* types. Methionine and cystine values reported here are probably low and should be treated with caution. From these data there appears to be no clear distinction between *Kabuli* and *Desi* cultivars based on their amino acid composition.

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References

1. Blagoveschenskii, A. V., *Biokhimiya*, 1960, **25**, 9.
2. Boulter, D., Thurman, D. A. and Derbyshire, E., *New Phytol.* 1967, **66**, 27.
3. Lal, B. M., Prakash, V. and Verma, S. C., *Experientia*, 1963, **19**, 154.
4. Jambunathan, R., and Singh, U., Proc. Internat Workshop on Chickpea improvement, ICRISAT, Hyderabad, India. 1979.
5. *Official Methods of Analysis*, Association of Official Analytical Chemists, 11th ed., Washington, D. C., 1970.
6. Singh, U. and Jambunathan, R., *J. agric. Fd Chem.*, (in press).
7. Danielson, C. E., *Biochem. J.*, 1949, **44**, 387.
8. Lowry, O. H., Rosebrough, N., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, 1951, **193**, 265.
9. Weber, K. and Osborn, M., *J. biol. Chem.*, 1969, **244**, 4406.
10. Narayana Rao, D., and Rajagopal Rao, D., *J. Fd Sci. Technol.* 1974, **11**, 139.

Analysis of Protein Patterns of an Edible Mushroom by Gel-Electrophoresis and its Amino Acid Composition

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Mature fruit-bodies (MF) of *Calocybe indica*, an edible white mushroom contain greater amount (17.21%) of protein than the buttons (15.07%) on dry wt. basis. Gel electrophoretic analysis of soluble proteins of fruit bodies of developmental stages reveals that 20, 18 and 16 protein bands are present in button (B), stipe with well differentiated pileus (SWP) and stipe with tiny pileus (SP), respectively. MF also shows 18 protein bands. Six to seven unique bands usually occur in B, SP and MF but only 3 have been detected in SWP. Certain bands which appear in one stage disappear in subsequent stages, while others remain unaltered. Chromatographic analysis of fruit-body protein shows that *C. indica* contains 12 amino acids and one amide. Glycine seems to be the predominant one.

The nutritive quality of edible mushrooms sometimes varies significantly with the species or strains. Proteins and amino acids are the most important constituents of fruit-bodies apart from other nutrients such as carbohydrates, vitamins and minerals. Considerable amount of work has been done on the estimation of amino acids and proteins of mycelia or fruit-bodies but relatively very little work has been done on qualitative and quantitative changes in proteins during morphogenesis of basidiocarps and protein patterns of edible fungi[1-3]. This investigation was done to study the changes in protein patterns during the development of fruit-body, protein content as well as amino acid composition of *Calocybe indica*, a popular edible mushroom of India[4-6].

Materials and Methods

Fruit-bodies were grown on soil-sand-wheat bran substrate to estimate proteins and amino acids. Fruit-bodies of different growth stages were collected, washed, dried at 105°C for 24 hr, powdered and stored in a desiccator until use. Thirty milligrammes of the dried powder were suspended in 6 per cent tri-chloro acetic acid (TCA) and heated for 20 min at 90-95°C to remove nucleic acid contaminants. The supernatant was decanted off and the pellet was washed twice with cold TCA (6 per cent) by centrifugation. The residue was digested with known volume of H_2SO_4 and copper catalyst[7]. Rest of the procedure was as described earlier[8].

For the analysis of protein patterns at different stages of fruit-body development, fresh fruit-bodies were collected from trays, homogenised with a known volume of Tris-HCl buffer solution in cold (4°C). Clear supernatants were collected after centrifugation at 10,000 × g in cold (4°C). This supernatant was again ultra centrifuged (1,05,000 × g) at 4°C for one hour. After ultracentrifugation, the clear supernatant was concentrated against 2M sucrose solution and stored in a deep freeze until use. Polyacrylamide gel electrophoresis of the concentrated material was done by following the procedure of Davis[9]. After electrophoresis, the protein gels were stained with 0.25 per cent coomassie blue and the Rf values of the protein bands were determined. For each treatment three replicates of gel column were used.

To analyse the amino acid composition of protein, fresh fruit-body was collected, washed and known quantity was homogenised with a known volume of NaOH (0.4N) in cold. Supernatant was obtained by centrifugation at 10,000 × g in cold. Protein was precipitated from the clear supernatant with 6 per cent TCA by adjusting its pH to 4.5. The precipitate was collected after centrifugation at 10,000 × g, for 15 min and washed twice with 6 per cent TCA. The remaining procedure was as stated earlier[8]. The amino acids in the acid hydrolysate of fruit-body were determined by paper chromatography[8].

Results and Discussion

Comparison of protein contents of fruit-bodies of developmental stages: Fruit-bodies of different stages viz., button (B); stipe with tiny pileus (SP); stipe with well differentiated pileus (SWP); stipe, pileus and gills i.e., mature fruit-body (MF) were collected from the trays and proteins were estimated.

The results in Table 1 show that mature fruit-body

TABLE 1. PROTEIN CONTENTS OF FRUIT-BODIES AT DEVELOPMENTAL STAGES

Stages of fruit-body	% protein content with ±S.E.*
Button (B)	15.07±0.44
Stipe with tiny pileus (SP)	13.13±0.59
Stipe with well differentiated pileus (SWP)	12.35±0.44
Mature fruit-body (MF)	17.21±0.58

*Based on 3 replicates and on dry wt basis.

of *C.indica* shows greater protein content than button or fruit-bodies of intermediate stages. The difference between mature and young sporocarps (stipe with tiny pileus) appears to be significant with regard to their protein contents. Apparently, button shows greater amount of protein than young sporocarps.

Comparative studies on protein patterns of fruit-bodies of developmental stages: To study the protein patterns of fruit-bodies of developmental stages, polyacrylamide gel electrophoresis technique of Davis[9] was adopted with modifications. The results reveal that 20, 18 and 16 protein bands are present in button, in stipe with well differentiated pileus and in stipe with tiny pileus, respectively (Fig.1). Mature fruit-body also shows 18 protein bands. The presence of common protein bands indicates the presence of common biochemical activities in different stages of development. There are certain unique protein bands found in a particular stage of fruit-body. These unique protein bands could be responsible for different types of tissue formation or expansion or both. Some bands are present in early stages but disappear in subsequent ones and reappear in later stages. All these variations both in number and type of protein probably contribute towards the importance and complexity of the particular stage of the fruit-body. The different protein patterns at different stages of development of a basidiocarp of *Volvariella volvacea* were studied by Chang and Chan.[10] They also reported different types of protein patterns in the fruit-bodies at developmental stages and suggested that the presence of a specific protein in a cell indicates the presence of a functioning gene and whatever is responsible for regulation of gene function is also responsible for cellular differentiation.

Amino acids of mature fruit-body: Since biological value of protein depends on the relative concentration of different amino acids, particualrly of the essential one, it was considered worthwhile to analyse the amino acid composition of fruit-body. The mature fruit-bodies were collected and their amino acids were determined. The results are shown in Table 2 along with those observed by others[11-14] in other edible mushrooms. Results of the chromatographic analysis of protein (acid hydrolysates) show that *C.indica* contains twelve

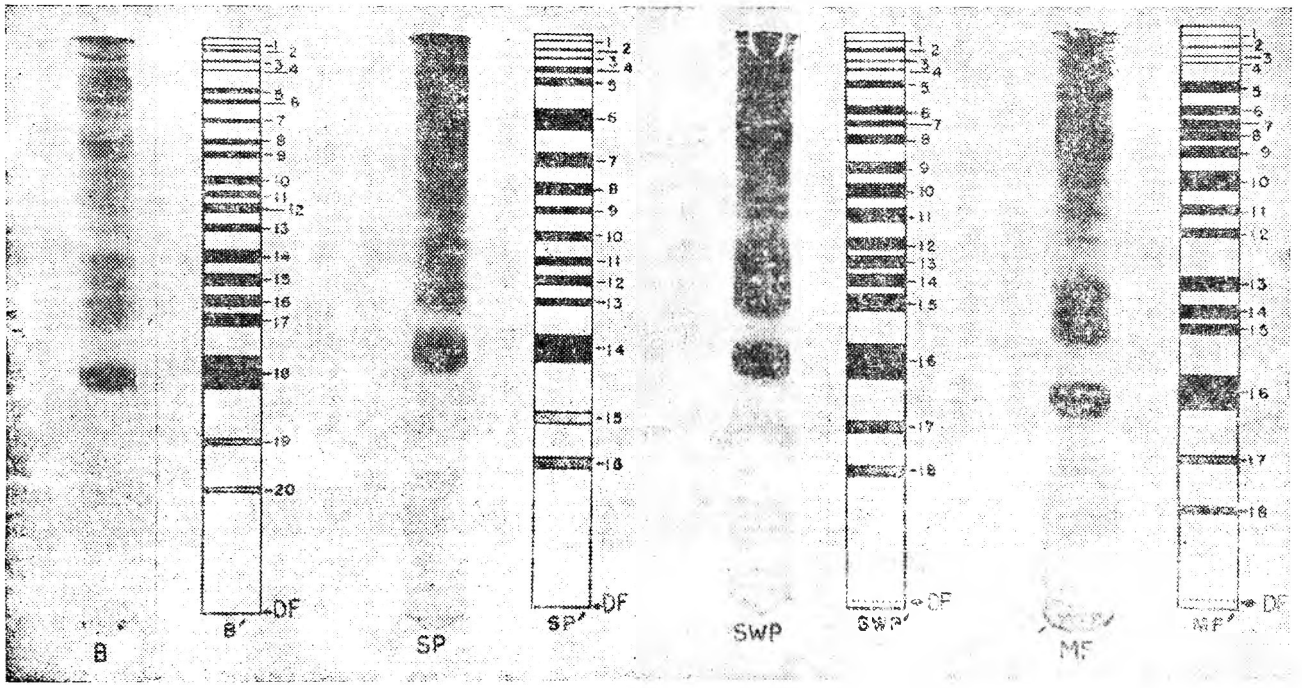

Fig. 1. Comparison of protein patterns of fruit-bodies of developmental stages.

B & B'—Button; SP & SP'—Stipe with tiny pileus; SWP & SWP'—Stipe with well differentiated pileus; MF & MF'—Mature fruit-body; Left-photographs; Right—diagrammatic sketches of the same. DF—Dye front.

TABLE 2. COMPARISON OF AMINO ACIDS (G. PER 100G PROTEIN) OF FRUIT-BODY PROTEIN OF DIFFERENT EDIBLE MUSHROOMS

Amino acids and amide	*Calocybe indica*	*Agaricus bisporus*[11]	*Pleurotus sajor-caju*[12]	*Volvariella diplasia*[13]	*Lentinus edodes*[14]
Aspartic acid	1.00	3.14	7.15	—	6.528
Threonine	0.52	1.48	3.53	4.208	4.352
Serine	—	1.89	3.31	—	4.336
Glutamic acid	2.12	7.06	17.92	—	22.464
Proline	Trace	2.50	2.80	—	3.616
Glycine	10.80	1.20	3.53	—	3.616
Alanine	1.60	2.40	4.43	—	5.072
Cystine	—	0.18	0.90	2.304	—
Valine	2.48	1.63	3.77	6.800	4.352
Methionine	—	0.39	1.31	0.896	1.456
Isoleucine	—	1.28	3.13	5.504	3.616
Leucine	—	2.16	4.94	3.504	5.792
Tyrosine	5.92	0.78	4.45	1.600	2.896
Phenylalanine	—	1.55	3.94	4.896	4.352
Histidine	2.40	0.64	1.57	2.096	1.456
Lysine	5.56	1.62	3.99	2.688	2.896
Arginine	Trace	1.90	4.41	4.096	5.792
Tryptophan	—	3.94	0.88	1.104	—
Glutamine	0.64	—	—	—	—
Hydroxyproline	1.76	—	—	—	—

*Based on three replicates All values are on dry wt basis.

amino acids and one amide. Of these, glycine appears to be the predominant one followed by glycine, tyrosine and lysine. Aspargine and proline are present in trace amounts. Of the 12 amino acids, 8 were identified earlier in the mycelia[8]. Isoleucine, phenylalanine, and methionine were identified only in mycelia[8] but not in the fruit-bodies. Since they have not studied the fruit-body amino acids, it would not be desirable to compare the results.

Acknowledgement

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References

1. Gilbert, T. F. A. and Robinson, R. F., *Econ. Bot.*, 1957, **11**, 126.
2. Worgan, J. T., *Progr. industr. Microbiol.*, 1968, **8**, 74.
3. Gray, W. D., *The use of fungi as food and in food processing*, Butterworths, London, 1970, 113.
4. Purkayastha, R. P. and Chandra, A., *Trans. Brit. Mycol. Soc.*, 1974, **62**, 415.
5. Purkayastha, R. P. and Nayak, D., *Mushroom J.*, 1977, No. 52, 132.
6. Purkayastha, R.P. and Nayak, D., *Taiwan Mushrooms*, 1979., **3**, 14.
7. Mahler, H. R. and Cordes, E. H., *Biological Chemistry*, Harper International edition, Harper and Row Publishers, New York, London, 1971, 1009.
8. Purkayastha, R. P. and Chandra, A., *J. Fd Sci. Technol.*, 1976, **13**, 86.
9. Davis, B. J., *Annals N. Y. Acad. Sci.*, 1964, **121**, 404.
10. Chang, S. T. and Chan, K. Y., *Mycologia*, 1973, **65**, 355.
11. Hayes, W. A. and Haddad, N., *Mushroom J.*, 1976, **40**, 104.
12. Jandaik, C. L. and Kapoor, J. N., *Mushroom J.*, 1976, **41**, 154.
13. Zakia, B., Srinivasan, K. S. and Singh, N. S., *J. Fd Sci. Technol.*, 1971, **8**, 180.
14. Doesburg J. J. and Meijer, A., *Voeding.*, 1965, **25**, 258.

Counteraction of Inhibitory Effects of Aflatoxin in *Bacillus megaterium*

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Among various normal cellular components tested, pyrimidine bases like thymine, cytosine and orotic acid were found to counteract the toxic effect of aflatoxin B_1 in growing cells of *Bacillus megaterium*. Addition of thymine to cells with aflatoxin has been found to counteract the inhibition of DNA, RNA and protein syntheses by 62%, 42% and 39% respectively. Reduction in cell elongation and restoration of cell multiplication are the other effects of counteraction. Analysis of base composition of nucleic acids of aflatoxin treated cells has revealed lower levels of thymine and cytosine and addition of thymine to cells with aflatoxin brings up the thymine and cytosine to normal levels.

Aflatoxin B_1 is the most toxic as well as carcinogenic, mutagenic and teratogenic fungal metabolite so far known. It is known to inhibit the synthesis of macromolecules in several biological systems. It has been demonstrated to some extent that metabolically activated aflatoxin can bind with macromolecules in rat liver both *in vivo*[1] and *in vitro*[2]. There is increasing evidence to support the theory that metabolic activation is necessary for many carcinogens including aflatoxin B_1 before they can react with cellular nucleophiles[3]. Though aflatoxin is known to inhibit the growth of several bacteria such activation of aflatoxin and its binding with macromolecules has not yet been demonstrated in them. However, the liver microsomal activated aflatoxin B_1 appeared to bind with t-RNA of *Salmonella typhimurium* T.A. 1530 and killed the bacteria and the addition of exogeneus t-RNA or DNA inhibited the lethal effect of the activated aflatoxin on the bacteria[4].

Aflatoxin occurs naturally in food and feed materials; several approaches have been made for preventing its production as well as for destroying and decontaminating the toxin. In the present studies an approach has been made to explore the possibility of counteracting the toxic effect of aflatoxin in a bacterial system.

Materials and Methods

A strain of *Bacillus megaterium* obtained from Type Culture Collections of National Chemical Laboratory, Poona, India, was used in the present investigations. A synthetic medium[5] was used for growth in presence of aflatoxin B_1 and nutritional factors such as yeast extract (Oxoid), casein (Hammerstein), casamino acids (Oxoid) and nucleic acid bases such as thymine (5 methyl-2,6-dihydropyrimidine from Calbiochem), cytosine (6-amino-2 hydroxypyrimidine from Calbiochem), guanine (2 amino hypoxanthine of E.Merck), adenine (6 amino purine of Hoffmann La Roche & Co.,) and uracil (2,6-dihydroxypyrimidine of BDH) with a view to study the counteraction of aflatoxin toxicity if any, by the nutritional factors in *B.megaterium*.

Aflatoxin B_1 was prepared by growing *A. flavus* (IMI 138325) on a medium designed by Basappa *et al*[6]. and extracting the 9-day old culture with chloroform and purifying by using alumina and silica gel columns and finally precipitating with petroleum ether (40-60°C). The precipitate was further purified by using Sephadex G 10 column using 2 per cent methanol in water as eluting solvent. The resultant product which gave a purity of 95 per cent was used in all the experiments in the form of ethanol solution.

Growth experiments: One day old culture of *B. megaterium* was inoculated (7×10^6 cells) into sterile 10 ml of the synthetic liquid medium contained in 50 ml conical flask added with aflatoxin B_1 at the concentration of 10μg/ml and nutritional factors at concentrations of 2 mg/ml. The flasks were incubated at 37°C on a rotary shaker (200 rpm) for 18 hr. The optical density was measured at 660 nm wavelength in Spectronic 20 as well as dry weight of cells were determined by drying the centrifuged and washed cells at 105°C for 6 hr in hot air oven. Standard curve prepared on optical density *vs.* dry weight was used throughout the studies. In certain cases, the number of cells were counted on Spencer Counting Chamber and the extent of elongation of cells was also measured under microscope.

Estimation of DNA in bacterial cells was carried out

by using diphenylamine reagent[7], RNA with orcinol reagent[8] and protein by Lowry's method[9] and expressed as μg/10 ml culture. Cell number of 2×10^9/10 ml was used as initial inoculum in the experiment. For determination of nucleic acid bases, the method of Marshak and Vogel[10] combined with TLC procedure of Randerath and Randerath[11] were used and expressed as μg of base/mg of dry bacterial powder, obtained after defatting and deproteinizing of bacterial cells. Average value of triplicates was recorded in all the experiments.

Results

It has been found that aflatoxin B_1 at sub-lethal levels induced cell elongation and inhibited cell division of *Bacillus megaterium*[3]; at higher levels growth as well as division completely stopped. Hence, to investigate the probable protective factors counteracting the effect of aflatoxin in the bacterium, several nutritional factors such as vitamins, proteins, amino acids and nucleic acid bases were examined. It was found that only thymine counteracted the toxic effect in terms of both cell multiplication and cell elongation as shown in Fig 1. However, the elongated cells as well as inhibited cells grew normally when transferred to fresh culture medium.

The optimum pH for growth of *B. megaterium* inhibition by aflatoxin B_1 and for counteraction by thymine was between 6 and 7. Hence pH 7.0 was used in all the experiments. If thymine is already present in the

TABLE 1. INFLUENCE OF PERIODICAL ADDITION OF AFLATOXIN OR THYMINE ON THE GROWTH AND MORPHOLOGY OF *B. MEGATERIUM*

Thymine already present

Aflatoxin B_1 added at (hr)	% elongated cells	Av. length of cells (μ)	Wt. of dry biomass (mg/10 ml)
0	26.8	3.0	3.4
2	17.5	2.8	3.6
4	18.4	2.5	3.8
6	17.3	2.6	4.1
8	9.9	2.6	3.8
Control	0.0	2.4	7.2
Aflatoxin (control)	75.5	5.3	0.5

Aflatoxin B_1 already present

Thymine added at (hr)	% elongated cells	Av. length of cells (μ)	Wt. of dry biomass (mg/10 ml)
0	18.9	2.7	3.3
2	23.0	2.7	3.4
4	27.3	3.4	3.5
6	49.4	4.5	2.2
8	68.1	4.8	0.5
Thymine (control)	0.0	2.7	5.4

All the observations were made after 18 hr of incubation at 37°C on rotary shaker.

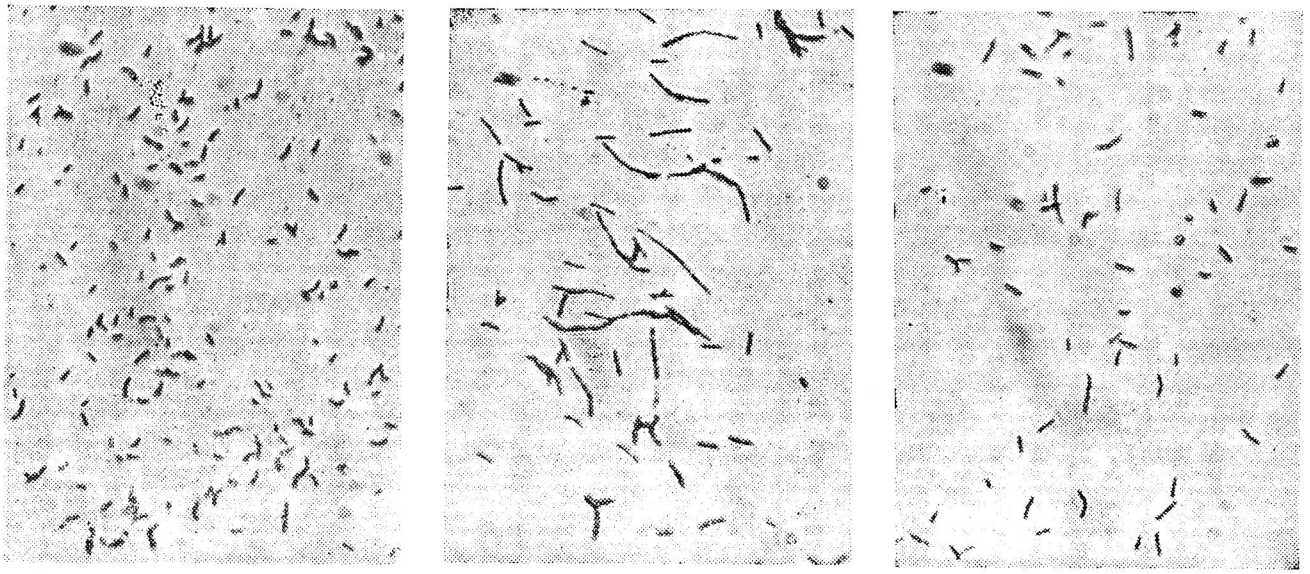

Fig. 1. Microscopic observations (x650) of crystal violet stained cells of *Bacillus megaterium* after growing in 10 ml synthetic medium with an inoculum of 7×10^6 cells in 50 ml conical flask in a rotary shaker for a period of 18 hr at 37°C.

A. Control—showing normal cells. B. Aflatoxin B_1 10 μg/ml added before inoculation—showing abnormally elongated cells. C. Aflatoxin B_1 10 μg/ml + thymine 2 mg/ml added before inoculation—note the counteraction of elongated cells as a result of the addition of thymine.

TABLE 2. COUNTERACTION OF AFLATOXIN TOXICITY BY VARIOUS PURINE AND PYRIMIDINE BASES

Bases	Dry wt. of biomass (mg/10 ml)
Control	7.5
Thymine	3.5
Cytosine	3.8
Orotic acid	4.0
Uracil	0.6
Adenine	1.2
Guanine	1.8
Aflatoxin control	0.6

1.5 mM/ml of each of the bases along with aflatoxin B_1 (10 μ g/ml) were used and observed after 18 hr of incubation with the bacterial cells.

TABLE 4. NUCLEIC ACID BASE COMPOSITION OF AFLATOXIN B_1 TREATED AND AFLATOXIN B_1 + THYMINE TREATED CELLS OF *B. MEGATERIUM*

Treatment	Base composition (μ g/mg) of dry powder*				
	Adenine	Guanine	Cytosine	Thymine	Uracil
Control	16.0	22.0	21.0	10.4	13.0
Aflatoxin B_1	25.0	43.0	18.3	7.6	16.0
Aflatoxin B_1 + Thymine	17.0	24.3	21.4	12.4	10.4

*Defatted and deproteinised bacterial cell mass obtained after 18 hr of incubation.

Aflatoxin B_1 concentration, 10 μ g/ml; Thymine concentration, 2 mg/ml.

Average of triplicate analysis.

medium, the sub-inhibitory levels of aflatoxin do not interfere with growth of the bacterium. Conversely. if aflatoxin is added first to the medium, its inhibitory effect could be counteracted by thymine only if the latter was added within 4 hr of incubation. Beyond this period the addition of thymine was ineffctive (Table 1). Even cytosine counteracted the effect of aflatoxin to the same extent as that of thymine. However, uracil, another pyrimidine base, and purine bases did not counteract the effect of aflatoxin in the bacterium as shown in Table-2.

Mitomycin-C another inhibitor of DNA synthesis was used at sub-lethal levels (0.01 μ g/ml) in growing cells of *B. megaterium*. Though abnormally elongated cells were present in the experimental medium, no counteraction could be obtained by thymine. This suggests that the mode of action of aflatoxin is probably different from that of Mitomycin-C.

Since thymine, orotic acid and cytosine counteracted the effect of aflatoxin in growing cells of the bacterium, it was attempted to find out the other associative changes to understand the mechanism of action of thymine in counteracting the inhibitory effect of aflatoxin. It was found by the periodical analysis (Table 3) that in the presence of aflatoxin B_1 synthesis of DNA was decreased by 95 per cent, RNA by 60 per cent and protein by 75 per cent at the end of 9 hr. The addition of thymine to the above system counteracted the inhibition of macromolecular synthesis to the extent of 62 per cent in the case of DNA, 42 per cent in RNA and 39 per cent in protein as shown in Table 3.

To rule out the possibilities of aflatoxin directly binding with purine and pyrimidine bases, aflatoxin was mixed with the bases at the molar ratio of 1:100 *in vitro* and absorption spectrum was determined.

TABLE 3. MACROMOLECULAR SYNTHESIS IN *B. MEGATERIUM* IN PRESENCE OF AFLATOXIN B_1 AND AFLATOXIN WITH THYMINE AT PERIODICAL INTERVALS

Time (hr)	DNA (μ /10 ml)			RNA (μ g/10 ml)			Protein (μ g/10 ml)		
	Control	Aflatoxin	Aflatoxin + thymine	Control	Aflatoxin	Aflatoxin + thymine	Control	Aflatoxin	Aflatoxin + thymine
0	180	180	180	360	360	360	840	840	840
3	265	180	200	680	480	500	1020	900	920
6	500	200	350	840	680	760	1600	1000	1140
9	600	200	450	1560	840	1140	2260	1200	1600
%inhibition at 9 hr	0.0	95	36	0.0	60	35	0.0	75	46
% counteraction at 9 hr	—	—	62	—	—	42	—	—	39

Initial cell number at 0 hr: 2×10^8 cells/ml. Aflatoxin concentration 50 μ g/ml. Thymine concentration 6 mg/ml.

Spectral characteristics of thymine and aflatoxin remained unchanged. This was also verified by TLC by looking to a single spot of aflatoxin-thymine complex other than that of aflatoxin alone and confirmed that there was no binding of aflatoxin with thymine.

The data in Table 4 show less of thymine and to some extent cytosine in nucleic acids when the growing cells were treated with aflatoxin. On the other hand, the levels of purine bases increased. But when thymine was added to aflatoxin treated culture there was an evidence of almost equal levels of thymine as well as cytosine in the nucleic acids indicating the incorporation of and counteraction by thymine.

Discussion

Aflatoxin B_1 is known to interact with DNA and RNA and bring about mutational changes, in microbes and carcinogenic changes in animals. There is enough evidence to indicate, that aflatoxin B_1 or perhaps its metabolites directly modify the template rather than having a direct effect on RNA-polymerase. It has also been found that activated metabolite derived from aflatoxin B_1 binds with DNA, yeast t-RNA, Poly G and Poly A[2].

It has been found that pyrimidine bases except uracil counteract the effect of aflatoxin in *B. megaterium* both in reducing the cell elongation and promoting cell division. The elongation of cells in presence of aflatoxin is believed to be due to interference of toxin with the synthesis of cell wall[12]. However, this view has been contradicted by a subsequent report[13]. The fact that uracil does not counteract suggests that aflatoxin does not directly interfere with RNA synthesis. But it is quite evident that DNA synthesis is directly affected. Since direct complexing of aflatoxin with thymine or any other pyrimidine or a purine base is ruled out, the inhibitory effect or its counteraction may either be due to an interference with thymine biosynthesis or due to competition between aflatoxin and thymine for the same site on the DNA strand. This may explain the ability of thymine to counteract the inhibitory effect of aflatoxin on *B. megaterium*. The results presented here show that mitomycin-C, an inhibitor of DNA synthesis[14] is different from aflatoxin in its mode of action although, there is some similarity on the morphological abnormality caused by the two compounds. While the toxic effect of aflatoxin is reversible, that of mitomycin-C is irreversible. Further work is in progress to elucidate the mode of action of aflatoxin and the protective action of thymine in the bacterium.

References

1. Swerson, D. H., Miller, E. C. and Miller. J. A., *Biochem., Biochem. Biophys. Res. Commun.*, 1974, **60**, 1036.
2. Garner, R. C., *Chem. Biol. Interact.*, 1973, **6**, 125.
3. Heidelberger, C., *Fed. Proc.*, 1973, **32**, 2154.
4. Garner, R. C., Miller, E. C. and Miller, J. A., *Cancer Res.*, 1972, **32**, 2058.
5. Mangalo, R. and Wachsman, T. T., *J. Bacteriol.*, 1962, **83**, 27.
6. Basappa, S. C., Jayaraman, A., Sreenivasa Murthy, V. and Parpia, H. A. B., *Indian J. exp. Biol.*, 1967, **5**, 262.
7. Dische, Z. in *Nucleic Acids*, edited by Chargaff, E. and Davidson, J. N., Academic Press, New York, 1955, Vol. I, 285.
8. Herbert, D., Phipps, P. J. and Strange, R. E., in *Methods in Microbiology*, Edited by Norris, J. R. and Ribbons, D. W., 1971, 289.
9. Lowry, O. H., Rosebrough, N. J., Lewis Farr, A. and Randall, R. J., *J. biol. Chem.*, 1951, **193**, 265.
10. Marshak, A. and Vogel, H. J., *J. biol. Chem.*, 1951, **189**, 597.
11. Randerath, E. and Randerath, K., in *Methods in Enzymology* edited by Lawrence Grossman and Kivie Moldane, 1967, Vol. XII Part A, 323.
12. Lillehoj, E. B., Ciegler, A. and Hall, H. H., *J. Bacteriol.*, 1967, **93**, 464.
13. Burmeister, H. R. and Hesseltine, C. W., *Bacteriol. Proc.*, 1967, 17 (Abst. A. 97).
14. Shiba, S., Terawaki, A., Taguchi, T. and Kawamata, J., *Nature, Lond.*, 1959, **183**, 1056.

Studies on Dehydration of Mushrooms (*Volvariella volvacea*)

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Dehydration conditions for *Volvariella volvacea* mushrooms were standardised using discrete mushrooms, after pretreatments leading to quality impairing polyphenol oxidase. An acceptable product was obtained in 90 min when dried in a fluidised bed dryer at 80°C with 7 kg batches. Dehydrated mushrooms when stored below 60% RH at 25°C remained acceptable.

Mushrooms are soft textured and highly perishable; dehydration, canning and freezing have been found suitable for preservation. Dehydration being cheaper is employed on commercial scale. The extent of drying of *Agaricus bisporus* is negligible[1]. India exported about 22,000 kg of mushrooms during 1973–74 earning foreign exchange of about 50 lakh rupees[2].

In recent years, sorption properties are receiving increasing attention. These are of considerable significance for systematic studies on packaging requirements and storage characteristics of foods in general and dehydrated foods in particular [3,4]. ERH studies in conjuction with adsorption and desorption isotherms will give direct information on the storage stability of the product and the water activity that will best correlate with the desired stability. In the present investigation an attempt has been made to dehydrate *Volvariella volvacea* cultivated under controlled conditions on paddy straw beds. Storage studies were also undertaken.

Materials and Methods

The culture of *Volvariella volvacea* was obtained from Tamilnadu Agriculture University, Coimbatore, and maintained in laboratory as paddy straw spawns. Mushrooms were cultivated in the laboratory on paddy straw[5,6] and those with sporophores fully opened and without any change in colour of gills were selected for dehydration. They were dehydrated as such as well as after various pretreatments and their effects on reconstitutional properties were ascertained.

The pretreatment was necessary to inactivate polyphenoloxidase (PPO) in mushrooms. The pretreatments tried were sulphiting (potassium metabisulphite solution of different strengths for 15 min, with a 1:10 proportion of mushrooms to metabisulphite solution), steam blanching and water blanching. The method of Ponting and Joslyn[7] was used with slight modification to follow the activity of PPO on the basis of rate of formation of coloured product from catechol or p-cresol.

Estimation of PPO activity: The assay was conducted by adding 2 ml of 0.018M catechol or p-cresol solution at a time to 0.5 ml of enzyme solution, in a total volume of 5 ml made with 0.005M phosphate buffer of pH 6.0 and 5.3 for catecholase and cresolase activities respectively. The reaction mixture was shaken and colour was read at 420 nm in Klet-Summerson photoelectric colorimeter after 3 min.

Dehydration of mushrooms: Mushrooms were dehydrated using tray dryer at 60±1°C, 70±1°C and 75±1°C. The air velocity in tray dryer was 700 ft/min. Dehydration was also carried out in fluidised bed dryer at 60±1°C, 70±1°C and 80±1°C with air velocity of 600 ft/min and 7 kg loading capacity.

Drying curves: Data on drying curves were collected by using, a modified method for determination of moisture content at intermittent times as mushrooms vary in size. Mushrooms were subjected to pretreatments and the drained weight was noted. At this stage, the moisture content of mushrooms was determined. They were then subjected to drying in tray and fluidised bed dryer. The moisture content of partially dehydrated product was determined periodically during drying by sampling and drying to constant weight. From the data obtained, the amount of moisture per gram of dry matter was determined and plotted on a 3 cycle semilog paper against time.

The product was evaluated both in dried and rehydrated condition. The criteria for evaluation were dehydration ratio[8], residual SO_2[9], browning index,[10] final moisture content and rehydration ratio.

Packaging and storage studies: Mushrooms, sulphited and dried at 80°C in fluidised bed dryer were placed in polyethylene bag (250 gauge) kept unsealed in dessicators, each of which served as a constant humidity

chamber. Relative humidities of 20, 40, 60, 80 and 90 per cent were obtained in desiccators by using aqueous H_2SO_4 solutions.[11] The effect of these RH values was studied at 25±1°C and 37±1°C. The samples were removed periodically and analysed for moisture content, browning index and rehydration ratio. The final moisture contents (as is basis) were plotted against respective humidities and sorption isotherm obtained. The ERH values were obtained from the isotherm according to the method of Wink.[12]

The samples used for ERH were also used for storage studies over a period of 60 days and evaluated at different stages organoleptically as well as for moisture content, browning index and rehydration ratio.

Results and Discussion

The studies on effect of blanching treatment on inactivation of PPO (Table I) revealed that blanching in boiling water required 3 min to inactivate the enzyme while it required 7 min with live steam. Steam blanching though prolonged was preferable as the mushrooms retained their texture during blanching, whereas the texture was lost in water blanching. Many workers have recommended steam blanching as pretreatment for mushrooms.[13–15]

It is known that SO_2 besides acting as a preservative also allows for higher temperature for dehydration. It is clear from the results (Table 2) that mushroom PPO was completely inactivated at 2000 ppm of SO_2. The colour and flavour of sulphited mushrooms were superior to steam blanched mushrooms. To retain sufficient amount of SO_2 in dehydrated product a pretreatment of 5000 ppm was used.

TABLE 1. EFFECT OF BLANCHING ON POLYPHENOLASE ACTIVITY

Blanching method	Time (min.)	% inactivation	
		Catecholase	Cresolase
Water	0	0	0
	1	43.68	25.00
	2	81.60	100.00
	3	100.00	—
Steam	0	0	0
	2	20.23	46.30
	4	53.95	88.47
	6	81.32	100.00
	7	100.00	—

TABLE 2. EFFECT OF SULPHITING ON MUSHROOM POLYPHENOLASE ACTIVITY

SO_2 Concn. (ppm)	% inactivation	
	Catecholase	Cresolase
0	0	0
500	30.49	33.30
1000	68.24	75.00
1500	85.89	100.00
1800	96.47	—
2000	100.00	—

Effect of temperature on drying rate: Fig. 1, 2, 3 and 4 represent the effect of drybulb temperature on drying rate of blanched, sulphited and untreated (mushrooms with no treatment) mushrooms in tray dryer and fluidised bed dryer. It is evident from the Fig. 1, 2 and 3 that during initial stages of drying, temperature did not have significant effect on moisture evaporation at 70° and 75°C. In fluidised bed dryer, almost same results were obtained for 60°, 70° and 80°C. It can be concluded from these results that in low moisture range, drying rate was significantly higher at higher temperature. This may be due to the redistribution of internal moisture

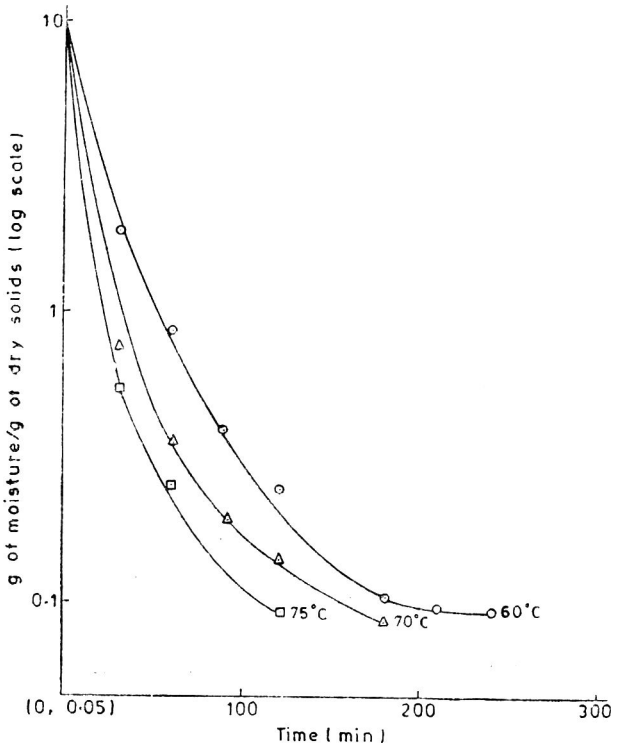

Fig. 1. Drying curves for tray dried, untreated mushrooms.

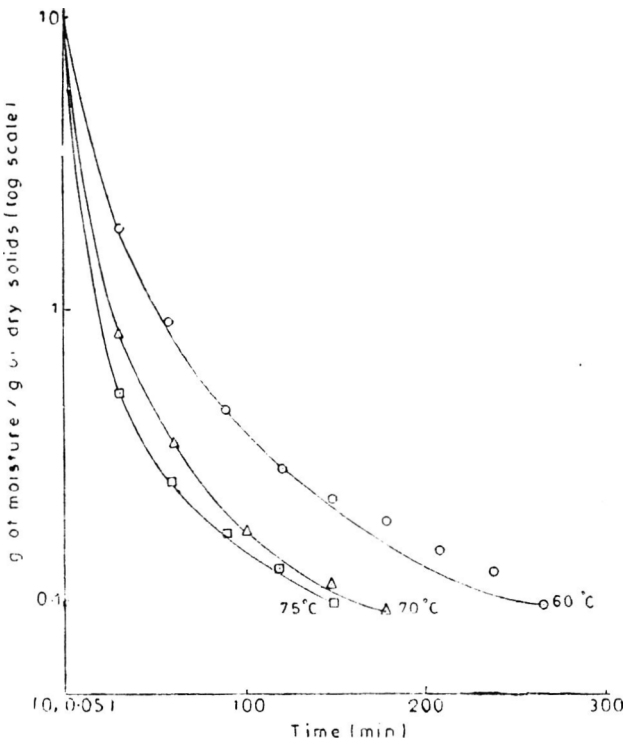

Fig. 2. Effect of Steam Blanching on Drying Rate of Tray Dried Product.

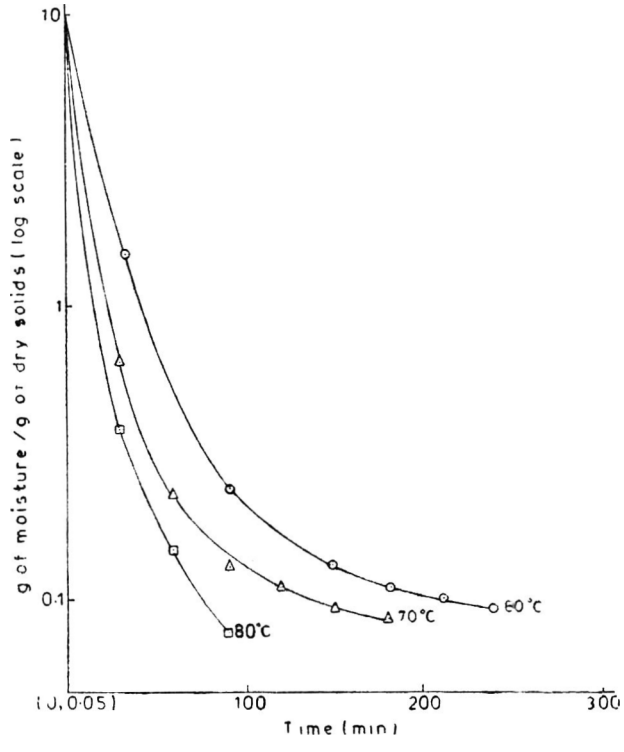

Fig. 4. Effect of SO_2 Treatment on Drying Rate n Fluidised Bed Dried Product.

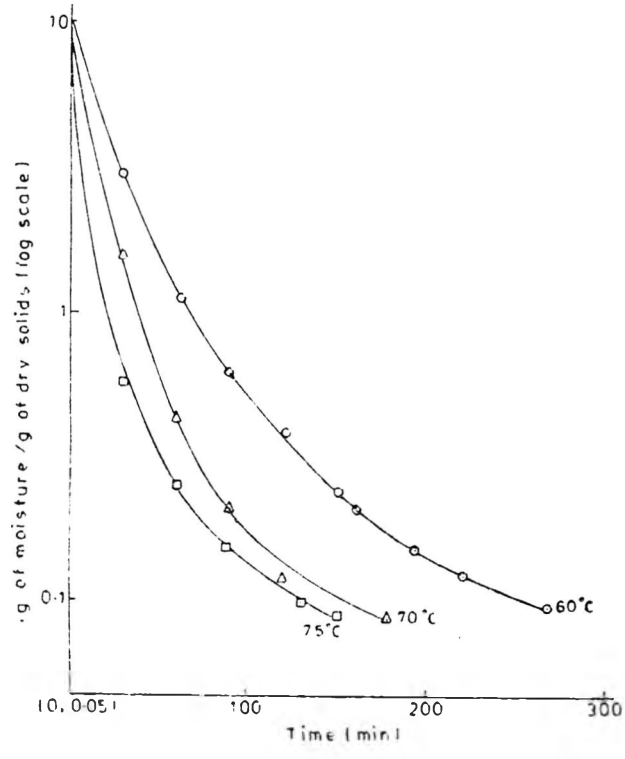

Fig. 3. Effect of SO_2 Treatment on Drying Rate of Tray Dried Product.

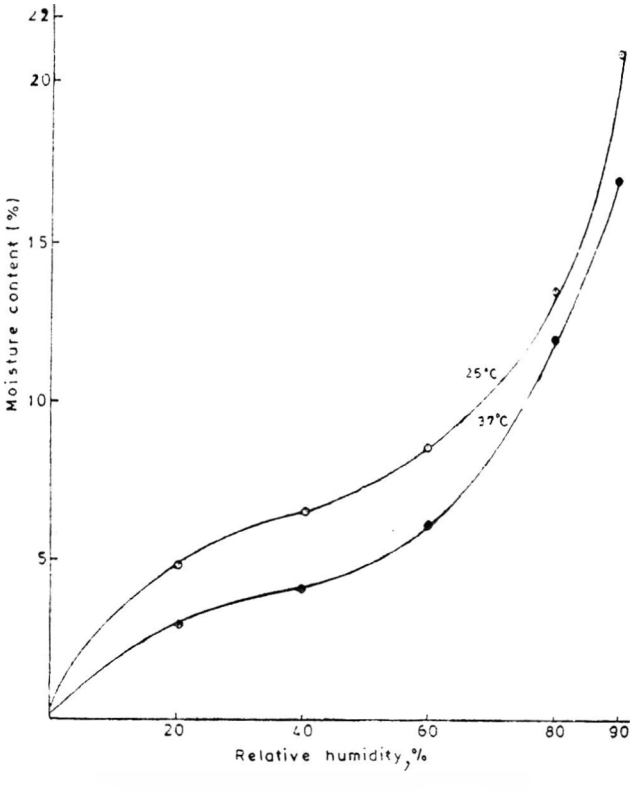

Fig. 5. Sorption Isotherm for Fluidised bed dried Mushrooms at 80°C.

TABLE 3A. DEHYDRATION CHARACTERISTICS OF MUSHROOMS

Treatment/ Blanching	Drying temp. (°C)	Dryer type	Dehydration time (min)	Final moisture (%)	Dehydration ratio	Browning index (O.D.)	Residual SO_2 (ppm)	Colour	Flavour	Texture
SO_2	60	FBD	270	9.10	10.63	0.19	1192	Good	Good	Crisp
SO_2	70	FBD	180	8.00	12.50	0.16	1210	Good	V.G.	Crisp
SO_2	80	FBD	90	5.50	11.28	0.16	1275	Attractive	Excellent	Crisp
SO_2	60	T.D.	270	8.76	12.00	0.21	1136	Good	Good	Crisp
SO_2	70	T.D.	180	9.08	12.70	0.18	1165	Good	Good	Crisp
SO_2	75	T.D.	150	8.91	11.22	0.16	1276	V.G.	V.G.	Crisp
Steam	60	T.D.	270	8.58	11.60	0.18		Gills blackening	Fair	Tough
Steam	70	T.D.	180	8.35	11.98	0.15		,,	Good	,,
Steam	75	T.D.	150	8.40	11.15	0.13		,,	Good	,,
Control	60	T.D.	240	8.09	12.10	0.45		Unpleasant	Poor	Soft
Control	70	T.D.	180	8.25	12.12	0.41		,,	Fair	Soft
Control	75	T.D.	120	8.15	12.00	0.40		,,	Fair	Soft

FBD—Fluidised bed dryer; T.D.—Tray dryer; V.G.—Very good

which is rate determining factor in this phase as the material attains the drybulb temperature of air during this region of drying and high temperature of material could be an accelerating factor for internal movement of moisture. Similar observations were made by other workers on potatoes.[16] From the drying curves it is evident that constant rate period lasted for very short time followed by extended falling rate period as has been reported by Saravacos[17] for other food material.

Effect of pretreatments on drying rate: It is clear from Fig. 2 that steam blanched mushrooms showed higher drying rate over sulphited and untreated mushrooms at the same temperature in the intial period of drying but in the falling rate period. Steam blanched mushrooms showed a slower rate of drying than that of sulphited mushrooms. The drying rate of sulphited mushrooms throughout drying was uniform at 60°, 70° and 75°C while drying rate of untreated mushroom was higher in the final stages of drying.

TABLE 3B. REHYDRATION CHARACTERISTICS OF MUSHROOMS

Treatment/ Blanching	Drying temp. (°C)	Dryer type	Dehydration time (min)	Rehydration ratio	Final moisture M_R	Coeff of rehydration C_R	Colour	Texture	Flavour
SO_2	60	FBD	270	4.8	81.1	52.8	8	7	7
SO_2	70	FBD	180	5.6	83.6	57.3	8	7	7
SO_2	80	FBD	90	5.7	83.4	60.3	9	9	9
SO_2	60	T.D.	270	4.7	80.6	51.5	8	7	7
SO_2	70	T.D.	180	5.1	82.2	56.2	8	7	7
SO_2	75	T.D.	150	5.3	82.8	58.2	8	7	8
Steam	60	T.D.	270	4.4	79.5	48.7	7	6	7
Steam	70	T.D.	180	5.0	81.7	54.5	7	6	7
Steam	75	T.D.	150	5.0	81.9	54.0	7	6	7
Control	60	T.D.	240	4.9	81.1	52.9	2	5	6
Control	70	T.D.	180	5.1	82.0	53.3	2	4	6
Control	75	T.D.	120	5.2	82.4	56.8	2	4	6

Points given for organoleptic characteristics were out of 10.
FBD—Fluidised bed dryer; NT—No treatment; T.D.—Tray drier; SB—Steam blanched

Dehydration characteristics: From the data in Table 3A it can be seen that mushrooms when dried at 80°C in fluidised bed dryer required only 90 min to attain 5.5 per cent moisture while it required 270 min at 60°C and at this stage moisture content was 9.1 per cent. The dehydration ratio was in the range of 10.63 to 12.7. The quality of the dehydrated product which received no treatment was found to be undesirable. The texture was soft and the product crumbled readily while browning was maximum.

In mushrooms blanched with steam, the texture of the dehydrated product was found to be very tough which lowered its acceptability. This is supported by results obtained by other workers[8] that mushrooms dehydrated with steam blanching yield products which are very tough and inferior in texture, flavour and colour.

Rehydration characteristics: The rehydration ratio (Table 3B) was in the range of 4.45–5.7. Rehydration ratio of mushroom was 5.7 for samples dried at 80°C in fluidised bed dryer and this was maximum among all other samples. Also these samples retained very good colour, flavour and texture after rehydration. The product had very attractive appearance.

Packaging and storage studies: The nature of the equilibrium adsorption isotherm obtained at 25° and 37°C was of sigmoidal type. They are characterised by inflection point which occur in the neighbourhood of 10-20 per cent relative humidity. It was observed that storage at high humidities stimulated moisture pickup resulting in deteriorative changes leading to off flavour and unacceptability of the product. From the storage data presented in Table 4, it is evident that samples stored

TABLE 4. STORAGE STUDIES OF FLUIDISED BED DRIED MUSHROOMS

Storage period (days)	Relative humidity (%)	Moisture (%)		Browning index (O.D.)		Rehydration ratio		Colour		Texture		Flavour	
		37°C	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C	25°C
15	20	4.0	5.0	0.14	0.12	5.8	5.6	V.G.	V.G.	Crisp	Crisp	V.G.	V.G.
	40	5.0	5.4	0.15	0.14	5.6	5.5	V.G.	V.G.	Crisp	Crisp	V.G.	V.G.
	60	6.0	8.2	0.17	0.15	5.2	4.0	V.G.	V.G.	Crisp	Crisp	V.G.	V.G.
	80	12.1	13.5	0.21	0.19	4.8	4.5	Good	Good	Soft	Soft	Slight off flavour	V.G.
	90	16.3	21.0	—	0.21	—	3.6	Dirty brown	Slight brown	V.Soft	V.soft	Off flavour	V.G.
30	20	3.0	5.1	0.16	0.13	5.5	5.0	V.G.	V.G.	Crisp	Crisp	V.G.	V.G.
	40	4.0	7.0	0.19	0.16	5.3	4.9	V.G.	V.G.	Crisp	Crisp	V.G.	V.G.
	60	6.0	8.5	0.23	0.18	4.8	4.5	Good	V.G.	Crisp	Crisp	V.G.	V.G.
	80	12.1	13.5	—	0.20	—	4.0	Brown	Slight brown	Soft	Soft	Off flavour	V.G.
	90	17.0	21.0	—	0.23	—	3.4	—	,,	—	V.soft	—	V.G.
45	20	3.0	5.3	0.18	0.15	5.0	4.8	V.G.	V.G.	Crisp	Crisp	V.G.	V.G.
	40	4.0	7.4	0.21	0.18	5.0	4.7	V.G.	V.G.	Crisp	Crisp	V.G.	V.G.
	60	6.2	8.7	0.25	0.21	4.6	4.4	Good	Slight brown	Crisp	Crisp	Good	Good
	80	12.4	13.7	—	0.24	—	3.9	—	,,	—	Soft	—	Good
	90	17.4	21.5	—	0.26	—	3.3	—	Brown	—	V.soft	—	Fair
60	20	3.0	5.4	0.20	0.18	5.0	4.6	V.G.	V.G.	Crisp	Crisp	V.G.	V.G.
	40	4.0	7.5	0.24	0.21	4.8	4.5	Good	Good	Crisp	Crisp	V.G.	Good
	60	6.4	8.3	0.28	0.23	4.6	4.4	Slight brown	Slight brown	Crisp	Crisp	Good	Good
	80	12.7	13.8	—	0.27	—	3.8	—	Brown	—	Soft	—	Fair
	90	17.9	21.6	—	0.29	—	3.2	—	Brown	—	V.soft	—	Poor

V.G. = Very good

at 37°C and 90 per cent RH developed off flavour and lost texture and were thus discarded. The moisture pickup in those stored at 37°C was less than that stored at 25°C and at all ranges of RH. Usually when the water relations are determined at number of temperatures, it is found that water content at any humidity increases with the fall of temperature. This is in agreement with the observation of earlier workers.[18,19]

Stored mushrooms. were evaluated organoleptically at 15-day intervals. The results showed that mushroom stored at higher temperature (37°C) and higher relative humidity developed off flavour while samples stored at 25°C showed no development of off flavour. There was no visible microbial growth on the product, when stored upto 2 months. It can be concluded from both ERH and storage studies that keeping the product at low RH of 10-20 per cent will be beneficial for retention of texture, colour and flavour.

References

1. Singer, R., *Mushrooms and Truffles*. Leonard Hill (Boons) Ltd., London, 1961, 5.
2. Anon., *Profodcil Bull.*, 1975, **9**, (1/2), 30.
3. Landrock, A. H., *Fd Technol., Champaign*, 1951, **5**, 332.
4. Taylor, A. A., *Fd Technol., Champaign*, 1961, **15**, 536.
5. Angle, R. Y., *Cultivation and Flavour Development in Mushroom Cultures*, Ph.D. Thesis, University of Bombay, 1976.
6. Chang, S. T and Hayes, W A., *The Biology and Cultivation of Edible Mushrooms*, Academic Press, New York, 1978 590.
7. Ponting, J. D. and Joslyn, M. A., *Arch. Biochem.*, 1948, **19**, 47.
8. Van Arsdel, W. B., Copley, M. J. and Morgan, A. I. (Jr)., *Food Dehydration* Vol I. 82, AVI Pub. Company, Westport, Connecticut, 1973, 82.
9. Potter, E. F., *Food Technol., Champaign*, 1954, **8**, 269.
10. Ramaswamy, S., *Chemistry and Technology of Vegetables*, Ph.D. Thesis, University of Bombay, 1974.
11. Wilson, W. E., *Ind. Engg. Chem.*, 1921, **13**, 326.
12. Anon., *J. Ass. off. Agric. Chem.*, 1966, **49**, 551.
13. Cruess, W. V. and Mrak, E.M., *Fruit Prod. J.*, 1942, **21**, 302.
14. Von Loesecke, H. W., *Drying and Dehydration of Foods*, Reinhold Pub. Corp., U.S.A., 1943, 113.
15. Mrak, E. M. and Ptaff, J., *Fd Technol., Champaign*, 1947, **1**, 147.
16. Ede, A. J. and Hale, K. C., Dept. Sci. ind. Research, *F. Invest Rep.*, **53**, 1948.
17. Saravacose, G.D. and Charm, S. E., *Fd Technol., Champaign*, 1962, **16**, 91.
18. Gane, R., *J. Soc. chem. ind.*, 1943, **62**, 139.
19. Benson, S. W. and Richardson, R. L. *J., Am. chem. Soc.*, 1955, **77**, 2585.

Ethyl Acetate as a Solvent for Extraction of Spice Oleoresins

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Laboratory studies on extraction efficiency of ethyl acetate to get oleoresin from pepper, chilli, ginger and turmeric have shown that it is comparable to ethylene dichloride and acetone in the yield of extractives and in quality. Ethyl acetate is safe and only sparingly soluble in water. It can, therefore, be considered as a suitable replacement for ethylene dichloride for oleoresin extraction. Ethyl acetate is inflammable as most other solvents and is liable to very slow hydrolysis.

Oleoresins, the extractives of spices are the important source of flavouring in a wide spectrum of products. The most commonly employed spice extracting solvent is ethylene dichloride[1-3] which is immiscible with water and safe against fire hazards. However, in recent years doubts were raised about the use of chlorinated solvents in food preparations. Alcohol is an innocuous solvent and acetone has the advantage of low boiling point. However, both are water miscible and hence the dilution of solvent occurs during repeated extractions. Besides water miscible non flavour substances such as carbohydrates, resins and gums also get extracted diluting the product. Moreover, acetone is said to leave a woody odour especially in the case of pepper oleoresin. Hexane or heptane can be a good solvent for extraction of essential oils and fats but the oleoresin obtained lacks body since more polar compounds do not generally get extracted. Carbon dioxide

because of the high volatility and consequent need for costly refrigeration has been used only to a very limited extent as an extracting solvent.

Though ethyl acetate is a recognized solvent, it has not been used for extraction of spices. This paper records the study carried out for extraction of some major spices and the possibility of substituting ethylene dichloride with ethyl acetate.

Materials and methods

Pepper, chilli, turmeric and ginger procured from local market were powdered and extracted by cold percolation with laboratory reagent grade ethylene dichloride, ethyl acetate and acetone under identical conditions.

The pungent principles piperine, capsaicin and gingerols were estimated by the method of Tausig *et al*[4], Mathew *et al*[5]. and Nambudiri *et al*[6] respectively. The ASTA method[7] was employed for estimation of curcumin and the colour from chilli was estimated by E.O.A. method[8].

Results and Discussion

Table 1 gives the results of analysis of various quality factors of spice oleoresin after extraction with ethylene dichloride, ethyl acetate and acetone. It can be seen that as an extracting solvent ethyl acetate is as good as ethylene dichloride. In the case of yield of oleoresin, while acetone has shown a marginal superiority, ethyl acetate is very slightly superior to ethylene dichloride. Acetone is clearly a better solvent for extraction of colour in chilli. All the three solvents are found to be comparable in the extaction efficiency of the active pungent principles. In pepper and ginger, ethyl acetate shows a slight superiority in extraction while in whole chilli ethyl acetate is slightly inferior to acetone and ethylene dichloride.

TABLE 1. ANALYSIS OF QUALITY FACTORS OF SPICE OLEORESINS OBTAINED WITH VARIOUS SOLVENTS

Spice	Ethylene dichloride	Ethyl acetate	Acetone
Pepper			
Extractives yield (%)*	6.2	6.7	7.4
Piperine (%) in			
Oleoresin	45	45.7	43.3
Spice	2.8	3.1	3.2
Chilli			
Extractives yield (%)*	10.5	10.8	10.6
Capsaicin (%) in			
Oleoresin	2.8	2.1	2.6
Spice	0.30	0.23	0.28
Colour value	17873	17385	22143
Ginger			
Extractives yield (%)*	4.1	4.2	4.5
Gingerols (%) in			
Oleoresin	20.1	22.0	20.1
Spice	0.82	0.92	0.90
Turmeric			
Extractives yield (%)*	7.0	7.2	7.7
Curcumin (%) in			
Oleoresin	31.9	31.8	31.1
Spice	2.2	2.3	2.4

*by cold percolation

Ethyl acetate is used in the manufacture of fruit flavours and perfumes and is recommended as an extracting solvent.[9] Table 2 gives the permitted level of some solvent residues. While the limits for acetone and ethylene dichloride are only 2 ppm, ethyl acetate is permitted upto as high as 250 ppm. Table 2 also gives a comparison of the properties and cost of solvents under study.

TABLE 2. GENERAL PROPERTIES AND IOFI LIMITS (IN FOOD AS CONSUMED) OF EXTRACTION SOLVENTS

Solvents	B.P. (°C)	Solubility in water (g/100 ml at 25°C)	Inflammability limit (vol % in air)		Flash point (°F)		Cost* (Rs./kg)	IOFI max residues allowed (ppm)
			Lower	Upper	Open	Close		
Ethylene dichloride	83.7	0.9	6.2	15.9	65-70	56	10.50	1
Ethyl acetate	77-77.2	7.4	2.18	11.5	30	24	9.25	250
Acetone	56.1-56.5	∞	2.15-3.0	11-13	15	0	12.50	2
Ethyl alcohol	78.4	∞	3.28	19	65	57	—	—
Hexane	66-71	0.014 at 15°C	1.25	6.9	-14.4	-7	—	—

*Chemical Weekly—Sewak Publications, Bombay, 6th May 1980.

Ethyl acetate is soluble in water to the extent of 7.4 g/100 ml at 25°C and forms an azeotrope with water which boils at 70.4°C containing 93 per cent ethyl acetate by weight. In most cases, extraction with water saturated solvent may not cause, a special problem. Simple drying agents can purify the solvent unlike in the case of alcohol and acetone where rectification is required.

Another possible objection to the use of ethyl acetate could be its slow hydrolysis to ethyl alcohol and acetic acid in presence of moisture. But both these products are quite safe. Since the hydrolysis is a slow process, the chance for this to occur is only in the solvent residue which is usually kept to a low level. The chances of esterification of the acid produced and trans-esterification of oleoresin constituents with ethyl acetate cannot be completely ruled out, though we feel that this will not be a problem as these will be present only in very negligible amounts. The odour characteristics of ethyl acetate are described as agreeable, mild and fruity. Hence it is hoped that the quality of the oleoresin will not be affected by the presence of traces of solvent and hydrolysed products. Moreover these spices are more valued for their non-volatile part except in the case of ginger and to some extent in pepper where both pungent principles and volatile oil are taken into consideration for quality. No adverse effect was noticed in these studies also. Ethyl acetate like most other solvents is inflammable and in this respect it will be inferior to ethylene dichloride and probably slightly superior to acetone. Cost wise ethyl acetate appears to have an edge over both ethylene dichloride and acetone.

References

1. Nambudiri E.S., Lewis Y.S., Krishnamurthy N., and Mathew A. G., *Flavour Ind.*, 1970, **1**, 99.
2. Mathew A. G., Lewis Y. S., Jagadisham R., Nambudiri E. S. and Krishnamurthy N., *Flavour Ind.*, 1971, **2**, 23.
3. Lewis Y. S., Mathew A. G., Nambudiri E. S. and Krishna-Murthy N., *Flavour Ind.*, 1972, **3**, 78.
4. Tausig, F., Suzuki J. I., and Morse R. E., *Fd Technol., Champaign*, 1956, **10**, 151.
5. Mathew A. G., Nambudiri, E. S., Ananthakrishna, S. M., Krishnamurthy N., and Lewis Y. S., *Lab Pract.*, 1971, **20**, 856.
6. Nambudiri, E. S., Mathew A. G., Krishnamurthy N., and Lewis Y. S., *Internat. Flavours Fd Additives.*, 1975, **6**, 135.
7. *Official Analytical Methods*, American Spice Trade Association, New York, 2nd Ed., 1968.
8. *EOA Specification and Standards*, No. 239. Essential Oil Association of USA, New York, 1965.
9. *Code of Practice*, International Organisation of the Flavour Industry, Geneva, Swizerland, 1979.

Packaging and Storage Studies of Deep-fat Fried Nendran Banana Chips

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Suitability of flexible packages and inert gas packing in sealed tins for the storage of fried 'Nendran' banana chips was investigated. It was found that for banana chips fried in fresh coconut oil, 300 gauge high density polyethylene and 400 gauge low density polyethylene bag packing are satisfactory up to two months while packing in tins under CO_2 is satisfactory up to six months at room temperature (28-32°C). The 'Nendran' banana chips fried in 'marvo' oil, a hydrogenated vegetable oil containing 0.02% BHA and packed in sealed tins under CO_2 were quite good up to 6 months whereas the chips fried in groundnut oil and packed under similar conditions were inferior in quality. Addition of turmeric powder as a natural colourant at 0.10 to 0.15% level during frying was found to enhance the colour of the chips which was stable up to six months in sealed tins under inert gas packing.

Banana chips manufactured from fully matured 'Nendran' variety banana (*Musa paradisiaca*) are popular in Kerala as snack food. The chips are manufactured throughout the year in Kerala. The consumers' preference for banana chips is based on colour, crispness, aroma and taste.[1,2] Chips sold in the markets are inferior in quality compared to fresh chips due to the lack of proper technique of packaging. Very little information is available on the packaging material suitable for banana chips. In the earlier communications, studies on the production of good quality banana chips[3] and determination of the optimum stage of maturity for the preparation of banana chips[4] were reported. The present investigation was undertaken to find out the suitable packaging material to improve the shelf life of banana chips.

Materials and Methods

Fully matured unripe bananas of 'Nendran' variety collected from a selected garden were peeled, steeped in 2 per cent salt solution for 15 min, wiped with cloth and 1.75 to 2 mm thick uniform slices were directly chipped into the coconut oil frying medium at 160°C, keeping fat and material ratio as 4:1. The banana chips were fried in batches of 500 g each. Two hand slicers were used simultaneously to chip the bananas quickly over the frying medium. The time taken between the start and end of frying was negligible. The temperature came down from 160°C to about 120°C after addition of the chips and slowly went up to about 140°C after 4 min towards the end of frying when salt was added at the rate of 0.6 per cent of the fresh material in the form of 20 per cent salt solution. The salt solution was sprinkled over the frying chips. After addition of salt about 1 min of frying was necessary to get a crisp product. Towards the end of frying the temperature was around 145-150°C.

The fried chips were packed in different packaging materials at the rate of 100g in 20 cm × 15 cm pouches of 400 gauge low density polyethylene (LDP-water vapour transmission rate g/m²/24 hr at 30°C and 90 per cent R.H.-2.8), 300 gauge high density polyethylene (HDP-water vapour transmission rate g/m²/24 hr at 38°C and 90 per cent R.H.–1.4) and in 18.1. square tins selaed under carbon dioxide (1.757 kg/cm²) after vacuumization (635 mm). Banana chips were also fried using "Marvo oil", a hydrogenated vegetable oil containing the antioxidant BHA at 0.02 per cent level and refined groundnut oil. The coconut oil used in these studies procured fresh from the factory was of good quality with no rancid smell and had a low F.F.A. (0.1-0.3 per cent) and peroxide value (0. to 0.2 per cent). The 'marvo oil' was procured from the manu-

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facturers and was of good quality. Refined groundnut oil was also of good quality with no rancid odour.

Addition of turmeric as a natural colourant to banana chips: Aqueous turmeric powder suspension in 3 different concentrations, viz. 0.05, 0.10 and 0.15 per cent were separately sprinkled along with 20 per cent aqueous salt solution towards the end of frying. The fried banana chips treated with turmeric were packed in similar conditions as detailed above.

Analytical techniques: Moisture, fat and total carotenoids were determined by following standard A.O.A.C. methods.[5]

Sensory evaluation of banana chips: Banana chips fried in coconut oil, packed in 300 gauge HDP, 400 gauge LDP, packed in tins under CO_2 and stored at room temperature (28-32°C) and 76 to 92 per cent relative humidity for 2,4, and 6 months were evaluated under standard conditions.[6] Banana chips packed under CO_2 in tins and stored at 0°C were given as reference standard. The evaluation of banana chips at regular intervals was carried out in two experiements. In the first, individual quality attributes were rated by multiple sample difference test and in the second, the overall quality was assessed on a 7-point hedonic rating test.[7,8] A discriminative communicative panel[6] was used for multiple sample difference test and an untrained panel for the hedonic rating test.

The quality description of desirable and undesirable aspects listed in Table 1 was arrived at after a few preliminary testing sessions.

In the multiple sample difference test, the panelists were asked to rate the quality attributes of the test samples compared to reference, as +5 is superior to reference (very large difference); O is equal to reference (no difference) and −5 is inferior to reference (very large difference). The reference standard was also given as one of the coded samples. The discriminative. communicative panel consisted of 20 scientific staff- The untrained panel consisted of 50 other members. They assessed the overall quality of chips on 7-point hedonic rating ranging from "like extremely" to "dislike extremely". The panelists were asked to rinse their mouth in between tasting different samples.

The scores for individual quality attributes and overall quality were analysed by Duncan's multiple range test.[8]

The effect of different frying media on moisture, fat, total carotenoids and the eating quality of banana chips packed in 18 l. square tins under CO_2 and stored for 6 months at room temperature were also studied. The quality evaluation of turmeric treated banana chips based on colour and appearance, texture, flavour and overall quality was done by a panel of trained judges. The scores of overall quality including the colour are given in Table 5.

TABLE 1. DESCRIPTION OF QUALITY ATTRIBUTES-BANANA CHIPS

Quality attribute	Desirable	Undesirable
Colour & appearance	Light yellow to light brownish yellow	Light brown Brown
	Bright	Bleached brown
	Uniform	Dull
		Not uniform
Texture	Brittle	Horny
	Crunchy	Gritty
	Crumbly	Soggy
	Crisp	
Flavour	Typica fresh aroma	Bitter rancid
	Balanced salt	Off aroma/taste
	Good after taste	Bitter/poor after taste

Results and Discussion

The deep-fat fried banana chips on prolonged storage and in exposed condition are subject to absorption of moisture, fading of the natural yellow colour and development of rancidity. Since initial trials showed that polyethylene bags upto 250 gauges were unsuitable even for a month's storage, flexible polyethylene of higher gauges and tin containers were chosen.

Crispness and colour of fried banana chips: The moisture and total carotenoids estimated for fresh and stored banana chips fried in coconut oil and packed in HDP and LDP are given in Table 2. The storage temperature was 26-32°C and the ambient humidity was 76-92 per cent R.H. The crispness of deep-fat fried chips depends on the moisture content. The results show that after 2 months' storage at room temperature the moisture content in banana chips packed in 300 gauge HDP increased to 3.8 per cent from the initial 3.2 per cent, while that of chips packed in 400 gauge LDP bags increased to 4.6 per cent. After three months' storage the moisture content in banana chips packed in

TABLE 2. MOISTURE AND TOTAL CAROTENOIDS IN STORED SAMPLES

Storage period (months)	Moisture (%)		Total carotenoids as β-carotene (μg/100 g)	
	HDP	LDP	HDP	LDP
Initial	3.2	3.2	3410	3410
2	3.8	4.6	3170	3080
3	4.4	5.5	—	—
4	5.1	5.8	2260	1980
6	6.5	6.5	1800	1600

HDP was 4.4 per cent while the chips packed in LDP had a moisture content of 5.5 per cent; this affected crispness of the product. The product lost crispness at 5 per cent moisture. The moisture content after 4 months storage was 5.1 per cent in HDP and 5.8 per cent in LDP. After 6 months' storage the moisture content was 6.5 per cent in both the packages. An increase in moisture content was observed on storage when the banana chips were packed in polyethylene bags which affected the crispness and in turn the overall quality of the product. The moisture content of 3.2 per cent remained unchanged in sealed tins.

The changes in natural colour of fried banana chips during storage were assessed by the changes in total extractable colour of fried banana chips packed in the above packages. After 2 months' storage at room temperature the total extractable colour as carotenoids, decreased to 3170 μg/100g in HDP and 3080 μg/100g in LDP bags from an initial value of 3410 μg/100g. The total carotenoid content after 4 months was reduced to 2260 μg/100g in HDP, 1980 μg/100g in LDP showing visually the fading/bleaching of colour in chips. The corresponding figures were 1800 μg/100g and 1600 μg/100g after 6 months' storage showing thereby that HDP was better than LDP for protecting the yellow colour where as, in sealed tin containers the depletion of colour was negligible, as it remained at 3170 μg/100g.

Sensory evaluation of banana chips: The results of sensory evaluation of stored banana chips for individual quality attributes and overall quality are given in Table 3.

2-*month storage:* In colour and appearance, all the test samples were rated as significantly inferior compared to the reference sample. In texture, 400 gauge LDP and 300 gauge HDP stored samples being comparable, were rated significantly inferior to reference or tin packed samples under CO_2. In flavour both LDP and HDP samples were rated significantly inferior to reference and tin packed samples.

A perusal of overall quality mean scores reveals that samples packed in 300 gauge HDP and those packed in tin under CO_2 were evaluated as "like moderately" while 400 gauge LDP samples were judged as "like slightly" for overall quality after a storage period of two months.

4-*month storage:* In colour and appearance the difference between samples was not very clear. CO_2 packed and reference samples being comparabel were rated significantly better than 400 gauge LDP and 300 gauge HDP samples. In overall quality, 400 gauge LDP and 300 gauge HDP samples were rated as "neither like nor dislike" while those packed under CO_2 in tins and reference samples were rated as "like moderately".

6-*month storage:* Reference or under CO_2 packed samples tin packed samples were comparable while the 300 gauge HDP sample showed significantly inferior colour and appearance, texture and flavour. In overall quality both LDP and HDP samples were comparable and were rated as "like slightly". CO_2 packed sample was scored as "like moderately".

The salient finding on an overall quality basis from the sensory evaluation of banana chips was that packing in polyethylene bags of 300 gauge HDP and 400 gauge LDP were reasonably satisfactory upto 2 months while packing in tin under CO_2 was satisfactory up to 6 months.

Effect of frying medium on the quality of fried banana chips: The results on quality of banana chips fried in coconut oil; marvo oil and refined groundnut oil and packed in 18.1. tins sealed under CO_2 (1.757 kg/cm^2) after vacuumization (635 mm) and stored at room temperature for 6 months are presented in Table 4. The

TABLE 3. SIGNIFICANCE OF QUALITY MEAN SCORES OF SAMPLES STORED FOR DIFFERENT MONTHS(m)

Quality attributes	LDP 400 gauge			HDP 300 gauge			Tins under CO_2			Reference*		
	2m	4m	6m	2m	4m	6m	2m	4m	6m	2m	4m	6m
Colour and appearance	-2.74^a	$+0.06^a$	NG	-1.15^b	-1.54^b	-2.61^a	-1.59^b	-1.08^{ab}	$+0.09^b$	-0.06^c	0.35^{ab}	$+0.04^b$
Texture	-1.81^a	-2.04^a	NG	-1.63^a	-2.77^b	-2.73^a	$+0.30^b$	-0.19^c	-0.12^b	$+0.15^b$	-0.08^c	-0.06^b
Flavour	-2.26^a	-2.33^a	NG	-1.28^b	-2.71^b	-2.88^a	-0.07^c	-0.19^c	-0.06^b	$+0.17^c$	-0.19^c	$+0.08^b$
Overall quality (max 7)	5.10^a	4.02^a		5.90^b	3.96^a	4.54^a	5.60^b	5.66^b	5.92^b	NG	5.85^b	NG

*Reference sample is packed in tins under CO_2 and stored at 0°C

Mean separation by superscripts for each attribute by Duncan's multiple range test, 5% level of significance

NG = not given

TABLE 4. QUALITY OF BANANA CHIPS PACKED UNDER CO_2 IN TINS—6 MONTHS STORED SAMPLES

Frying medium	Moisture (%)	Fat (%)	Total carotenoids as β-carotene (μg/100g)	Colour	Texture	Flavour
Coconut oil	2.7	31.3	3060	Yellow	Crisp	Good
Marvo oil	2.6	29.2	3200	Bright yellow	,,	,,
Groundnut oil (refined)	2.8	31.6	1100	Light yellow with a few bleached pieces	,,	Fair

banana chips fried in coconut oil were in good condition with respect to colour, crispness and flavour. The product had good yellow colour and the total carotenoid content was 3060 μg/100 g. The moisture and fat contents were 2.7 and 31.3 per cent repsectively. The banana chips fried in marvo oil possessed colour, crispness and flavour. The product had excellent yellow colour and the total carotenoid content was 3200 μg/100 g compared to the initial value of 3410 μg/100 g. This may possibly be due to the effect of BHA on the retention of colour. The fat content of 29.2 per cent was comparatively less compared to the chips fried in coconut oil. The moisture content was 2.6 per cent. The banana chips fried in refined groundnut oil were inferior in quality to chips fried in coconut oil and marvo oil. The chips were rather lighter in colour and a few pieces were also bleached while the quality also deteriorated. The moisture and fat contents were 2.8 and 31.6 per cent respectively.

Addition of turmeric as a natural colourant to banana chips: It is seen from Table 5 that after a period of 4 months' storage at room temperature, the total organoleptic score differed with the type of packaging material and the concentration of turmeric used. However, a suspension of turmeric of 0.10 to 0.15 per cent helped to retain the yellow colour whereas the chips without the addition of turmeric were subjected to fading of yellow colour and development of white streaks due to bleaching in a few isolated chips.

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TABLE 5. EFFECT OF ADDITION OF AQUEOUS SUSPENSION OF DIFFERENT CONCENTRATIONS OF TURMERIC POWDER ON THE STORAGE LIFE OF FRIED BANANA CHIPS PACKED IN FLEXIBLE PACKAGES AND IN TINS UNDER CO_2

Packages	Turmeric level (%)	Moisture (%)			Fat (%) initial	Total organoleptic score* (out of 100)	
		Initial	2M	4M		2M	4M
LDP – control	—	3.0	4.4	5.7	32.8	77	49
HDP – control	—	3.2	4.0	4.9	32.3	79	54
Tins – control	—	3.2	3.4	3.6	32.4	84	74
LDP	0.05	3.1	4.3	5.4	31.7	80	58
LDP	0.10	3.2	4.2	5.3	31.1	82	62
LDP	0.15	3.0	4.2	5.2	31.0	83	63
HDP	0.05	3.1	4.0	4.8	32.3	81	60
HDP	0.10	3.2	3.9	4.8	30.8	83	63
HDP	0.15	3.0	3.8	4.6	31.5	84	64
Tins	0.15	3.2	3.3	3.6	31.0	86	77
Reference	0.05	2.8	2.8	3.0	32.1	87	80
Reference	0.10	2.8	2.8	3.0	31.3	88	81
Reference	0.15	2.6	2.7	2.8	31.3	88	82

*Product appeared inferior in quality when the organoleptic score was < 55
Organoleptic rating: 55-60 Fair; 60-70 Good: > 70 Very good
Reference sample is packed in tins under CO_2 and stored at 0°C.

References

1. Jain, N. L., Nair, K. G, Siddappa, G. S. and Lal, G., *Food Sci., Mysore*, 1962, **11**, 335.
2. Godavari Bai, S. and Narayana Rao, M., *J. Fd Sci. Technol.*, 1969, **6**, 169.
3. Satyavati Krishnankutty, Bhat, A. V, George Varkey, A. Menon, K. G. K. and Mookerjee, K. K., *Proceedings of the Symposium on Fats and Oils*, Association of Food Scientists and Technologists, CFTRI, Mysore, 1978, 75.
4. Satyavati Krishnankutty, Bhat, A.V. and George Varkey, A., *J. Fd Sci. Technol.*, 1978, **15**, 68.
5. *Official Methods of Analysis*, Association of Official Agricultural Chemists, Washington, D. C., 11th Ed., 1970.
6. *Guide for sensory evaluation of foods, Part I. Optimum requirements*, IS: 6273 (Part I), Indian Standards Institution, New Delhi, 1971.
7. *Guide for sensory evaluation of foods, Part II. Methods and evaluation cards*, IS: 6273 (Part II), Indian Standards Institution, New Delhi, 1971.
8. Duncan, D. B., *Biometrics*, 1960., **16**, 671.

Effect of Dietary Fat on Deposition of Fat and Fatty Acid Composition of Tilapia *(Tilapia mossambica)*

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The effect of dietary fats on the visceral and body fat of the fish tilapia (*Tilapia mossambica*) was studied. Residual oil in groundnut cake, coconut oil, mustard oil and sardine oil were the sources of fatty acids in the experimental diets used. Feed containing coconut oil induced deposition of maximum amount of saturated fatty acids and lower fatty acids (C_6, C_8, C_{10}, C_{12}, C_{14}) in the visceral and body fat of fishes. Mustard oil induced deposition of maximum amount of polyunsaturated fatty acids followed by sardine oil. Deposition of $C_{22:1}$ acid in body as well as viscera was very significant in the case of fish fed on feed containing mustard oil. It is observed that dietary fatty acid pattern has got significant effect on the fatty acid composition of the fish.

With the increased tempo in aquaculture the nature and composition of fish feed have become a field of investigation of paramount importance. Fat is perhaps the most important source of energy for the fish for its various biological purposes. Seasonal changes in body fat were observed in fish depending upon factors like intensity of feeding, environmental conditions, spawning, etc[1-3]. Various types of fats were widely used by different workers as sources of dietary fat in feeds[4,5]. In this investigation four types of common fats namely, residual oil present in groundnut cake, coconut oil, mustard oil and sardine oil are used and their possible effect on the deposition, interconversion and nature of fatty acids deposited in the extractable lipids of muscle and viscera are investigated in detail. A common fresh water fish extensively cultured in the country tilapia (*Tilapia mossambica*) was subjected to detailed study for this purpose.

Materials and Methods

Fresh water fish tilapia just before spawning was brought to the laboratory and put in large tanks with plenty of water and continuous aeration. The fish were allowed to spawn and the young ones when they are one month old were separated and divided into 4 batches and put into 4 different tanks (60 fishes in 200 l. of water) with continuous aeration. The water temperature of the tank was kept at $27\pm1°C$. They were provided with different types of feeds (Feed I, II, III and IV as in Table 1). They were not allowed to breed as these tanks did not contain any male ones. After six months of growth they were taken out and analysed immediately.

Fish (20 numbers each weighing between 20 and 25 g) were caught and immediately beheaded. The viscera (intestinal portions with contents) were separated. Muscle was separated from the bones. The whole muscle was minced in a blender and 100g of minced fish meat was taken for lipid extraction. The entire viscera was taken for lipid extraction. The lipids were extracted from the muscle and viscera by the method of Bligh and Dyer[6] using chloroform-methanol (2:1 v/v) mixture successively with three volumes of solvent mixture (w/v)

TABLE 1. COMPOSITION OF FEEDS USED FOR FEEDING*

Feed ingredients	Moisture (%)	Ash (%)	Protein (%)	Carbohydrate (starch) (%)	Fat (%)
Feed I Groundnut cake + Tapioca starch	6.3	4.6	22.8	52.2	2.1
Feed II Feed I + Coconut oil	6.3	4.0	22.0	47.1	6.5
Feed III Feed I + Mustard Oil	6.2	3.9	22.1	47.0	6.4
Feed IV Feed I + Sardine oil	6.3	4.0	22.1	47.1	7.2

Vitamins added per kg of all finished feed samples: Thiamine hydrochloride, 250 mg; riboflavin, 50 mg; nicotinamide, 350 mg; vitamin B_{12}, 50 mg; folic acid; 25 mg; pyridoxine, 12.5 mg;, inositol 20 mg; vitamin A palmitate 10,000 I.U. and vitamin D, 1000 I.U.

*Period of feeding: 6 months.

until the residue is free from lipids. The lipids present in the chloroform layer were concentrated and dried under vacuum and kept in sealed ampules under nitrogen, in a deep freezer pending further analysis. The lipids were saponified in an atmosphere of nitrogen and fatty acids were separated and converted to their corresponding methyl esters by boron trifluoride-methanol reagent.[7] Fatty acids were analysed by gas liquid chromatography.

Gas-liquid chromatography: The methyl esters were analysed on a gas chromatograph using flame ionisation detector and strip chart recorder (10 mv) (Varian, Techtron) chart speed 30 cm/hr. The column used was stainless 6ft × ¼" (o.d.) packed with silar 10 C, 10 per cent on Anachrom ABS (110-120 mesh). Operating conditions were as follows: Column temperature 196°C, injection temperature 250°C. detecter temp. 275°C, carriergas nitrogen 40 ml/min. Identification and quantitation were done as reported earlier[8].

Analysis of fish feeds: The fish feeds were analysed for ash, moisture, protein and carbohydrates according to the methods of AOAC[9]. Lipids were extracted by the method of Bligh and Dyer[6]. Free sugar and glycogen were estimated by colorimetry using anthrone.[10] The feed composition is given in Table 1.

Results and Discussion

It is seen from Table 2 that Feeds II and IV caused maximum fat deposition in the body of tilapia. Fish which were fed with Feed I, which contained 2.1 per cent fat in groundnut cake as the only source of fat recorded the minimum of 5 per cent fat deposition in muscle.

Effect of dietary fats on fat content of viscera: The lipids were extracted from the viscera (only that portion of viscera containing intestines were taken) to study the nature of lipid breakdown in the intestine and its effect on the body fat composition. Viscera of fish fed with Feeds II and IV showed maximum fat. Fish fed with Feed I showed minimum fat. However, fish fed with Feed II (containing coconut oil) showed maximum visceral fat of 35.0 per cent.

Free sugar and glycogen: All four Feed samples showed almost a similar pattern in the amount of free sugars and glycogen in their body indicating that the different types of fat apparently showed little influence.

Effect of the amount of fat on body composition: Feed I caused the minimum fat deposition in the body (5.0 per cent). Feeds II, III and IV which contained about 6 to 7 per cent fat produced fish, after six months, with body fat in the range of 9 to 11 per cent.

TABLE 2. COMPOSITION OF FISH AFTER FEEDING FOR SIX MONTHS

Sample	No. of fishes	Moisture (%)	Ash (%)	Protein (%)	Body fat (%)	*Visceral fat (%)	Free sugar (mg/100 g)	Glycogen (mg/100 g)
Feed I	21	71.7	4.4	14.9	5.0	11.5	20.0	3.2
Feed II	53	69.4	3.4	14.0	10.2	35.2	25.0	2.5
Feed III	51	74.9	2.8	14.2	9.5	26.0	21.0	2.5
Feed IV	48	69.0	2.7	13.3	11.5	30.7	21.0	3.1

*Portion containing intestines with contents only.

TABLE 3. SATURATED FATTY ACID COMPOSITION OF THE BODY AND VISCERA LIPIDS AFTER FEEDING

Fatty acids	Feed I			Feed II			Feed III			Feed IV		
	Feed	Viscera*	Body	Feed	Viscera*	Body	Feed	Viscera*	Body	Feed	Viscera*	Body
C_6	–	—	—	—	2.3	2.3	—	1.4	—	—	2.5	—
C_8	—	0.4	0.1	2.8	0.8	1.9	—	0.3	—	—	0.2	—
C_{10}	0.2	0.3	0.2	4.5	—	0.8	—	0.4	—	—	0.3	0.4
C_{12}	0.2	0.8	0.2	14.0	6.8	6.6	0.3	0.6	0.5	—	0.3	1.2
C_{13}	—	1.8	—	—	0.4	—	—	—	0.1	—	—	0.3
C_{14}	0.3	3.3	2.8	13.0	10.3	8.5	0.2	1.9	1.6	7.0	6.9	8.9
C_{15}	—	0.9	0.9	—	0.7	0.9	—	0.6	—	0.4	—	1.1
C_{16}	13.8	18.4	16.4	12.0	17.1	15.7	8.5	11.8	12.6	20.0	17.1	17.8
C_{18}	3.7	6.1	6.9	2.8	5.6	5.4	2.43	4.0	5.2	4.4	5.3	5.0
C_{20}	1.8	—	—	0.4	2.6	—	1.8	5.2	4.3	0.9	—	0.3
C_{22}	4.5	—	—	—	—	—	1.7	2.1	2.3	0.7	—	—
C_{24}	2.3	—	—	0.5	0.1	—	1.9	0.6	—	0.9	0.8	1.7
Total	26.8	32.0	27.5	50.0	46.7	42.1	16.83	28.9	26.6	34.3	33.4	36.7

*Viscera includes intestines with contents.

Saturated fatty acids: Coconut oil containing fish feed (Feed II) induced maximum amount of saturated fatty acids (46.7 per cent) (Table 3). It is significant that the body and viscera contained higher amounts of C_6, C_8, C_{12} and C_{14} saturated fatty acids compared to fish fed on other feeds. The fish which were fed with Feed III (containing mustard oil) showed lowest level of C_{16} saturated acid (11.8 per cent) compared to others. Feed IV containing sardine oil induced maximum amount of C_{16} saturated acid (17.8 per cent) in the body. Of the higher saturated fatty acids, the feed containing mustard oil produced maximum $C_{20:0}$ acid in the fish. On the whole the body fat and visceral fat showed distinct similarity in their fatty acid composition.

Monounsaturated fatty acids: Fish feed containing sardine oil showed maximum amount of $C_{16:1}$ acid in the body (8.4 per cent) and visceral fat (8.9 per cent) (Table 4). However, mustard oil produced fish having the lowest level of $C_{16:1}$ acid (3.4 per cent). Feed I produced fish with maximum amount of $C_{18:1}$ acid in the body (39.4 per cent) and viscera (38.0 per cent) as the feed itself contained the highest amount of

TABLE 4. MONOUNSATURATED FATTY ACID COMPOSITION OF THE BODY AND VISCERA LIPIDS AFTER FEEDING

Fatty acids	Feed I			Feed II			Feed III			Feed IV		
	Feed	Viscera**	Body	Feed	Viscera**	Body	Feed	Viscera**	Body	Feed	Viscera**	Body
$C_{14:1}$	—	0.4 0.6*	0.7	—	—	—	—	—	0.4	0.2	0.9	—
$C_{16:1}$	0.9	5.8	6.7	0.2	6.6	6.5	0.4	2.8	3.40	9.6	8.9	8.4
$C_{18:1}$	39.5	38.0	39.4	31.40	31.3	32.7	29.3	29.0	29.2	20.4	32.6	28.5
$C_{20:1}$	0.3	3.1	3.1	0.2	—	2.2	3.1	—	—	0.2	1.9	2.1
$C_{22:1}$	0.3	—	0.9	0.8	—	—	10.8	7.2	5.3	1.1	0.3	1.0
$C_{24:1}$	0.4	0.9	0.5	—	0.7	1.5	1.6	1.9	2.0	—	—	—
Total	41.4	48.8	51.3	32.60	38.6	42.9	45.2	40.9	40.3	31.5	44.6	40.0

*Unidentified **Viscera includes intestines with contents.

TABLE 5. POLYUNSATURATED FATTY ACID COMPOSITION OF THE BODY AND VISCERA LIPIDS AFTER FEEDING

Fatty acids	Feed I			Feed II			Feed III			Feed IV		
	Feed	Viscera*	Body	Feed	Viscera*	Body	Feed	Viscera*	Body	Feed	Viscera*	Body
$C_{18:2}$	31.5	8.4	11.4	17.1	9.6	9.0	37.4	24.0	23.7	9.5	11.9	6.7
$C_{18:3}$		0.9	0.4		0.4	0.3	0.6	1.8	2.0	0.5	0.8	0.6
$C_{18:4}$		2.9	2.5		2.0	1.0	—	—	—	2.1	1.4	1.3
$C_{20:4}$		1.4	0.9		1.2	1.0		1.1	2.6	0.7	0.5	1.5
$C_{20:5}$		1.8	2.2		1.6	1.6		0.6	0.9	7.0	1.0	2.4
$C_{22:3}$		—	—		—	0.4	—	—	0.7	0.9	0.3	0.6
$C_{22:4}$		0.9	1.0		0.4	0.4		—	—	0.7	0.3	0.5
$C_{22:5}$		1.6	1.4		0.6	0.2		0.9	1.3	2.7	2.3	2.6
$C_{22:6}$		1.6	1.2		0.9	1.4		1.3	1.4	8.8	3.0	6.0
Unidentified		—	—		—	—		0.4	0.3	—	—	—
Total	31.5	19.5	21.0	17.1	16.7	15.3	38.0	30.1	32.9	32.9	21.5	22.2

*Viscera includes intestines with the contents.

this acid (39.5 per cent). Although Feed IV (containing sardine oil) contained only 20.0 per cent of $C_{18:1}$ (lowest among the four feeds) the body fat as well as the visceral fat showed apparently little difference compared to the other fish samples which were fed on diets having a different level of this acid. This clearly showed that the fish itself is capable of *de novo* synthesis of this fatty acid by extension of chain length from lower acids.

Among the higher monounsaturated fatty acids Feed III containing $C_{22:1}$ (10.8 per cent) induced highest levels of this acid in the body fat of the fish(5.3 per cent).

Polyunsaturated fatty acids: Feed III containing mustard oil induced maximum amount of polyunsaturated fatty acids in the body (32.9 per cent) and visceral fat (30 per cent) followed by Feed IV containing sardine oil (Table 5). Although Feeds I and III contained high amount of $C_{18:2}$ acid (31.5 and 37.4 per cent) respectively) compared to II and IV (17.1 and 9.5 per cent respectively), what is most significant is the fact that Feed III caused deposition of large amount of these acids both in body (23.7 per cent) and in visceral lipids (24 per cent) of the fish.

Feed IV which contained sardine oil (higher amount of $C_{22:6}$ acid (8.8 per cent) caused maximum amount of $C_{22:6}$ acid (6 per cent) in the body of the fish. As far as the fatty acid composition is concerned the diet is found to have significant effect on the composition of the body of the fish. On the whole it can be seen that the dietary fat has got distinct influence on the fatty acid composition of the fish. It can be seen that inclusion of marine fish fat like sardine oil favours the deposition of highly unsaturated fatty acids in the body fat of fresh water fish.

References

1. Laskar, R. and Theilacka, G. H., *J. Lipid Res.*, 1962, **3**, 60.
2. Lovern, J. A., *Oceanogr. Mar. Biol.*, 1964, **2**, 169.
3. Reiser, R., Stevenson, B., Kayama, M., Choudhury, R. and Hood, D. W., *J. Am. Oil Chem. Soc.*, 1963, **40**, 507.
4. Kelly, P. B., Reiser, R. and Hood, D. W., *J. Am. Oil Chem. Soc.*, 1958B, **35**, 503.
5. Braekkan, D. R., Lambertsen, G. and Andresen, J. Report on Technological Research Concerning Norweigian Fish Industry, Directorate of Fisheries, Norway, **5**, No. 5, 1971.
6. Bligh, E. G. and Dyer, W.J., *Canadian. J. Biochem. Physiol.*, 1959, **37**, 911.
7. Morrison, W. R. and Smith L. M., *J. Lipid Res.*, 1964, **5**, 600.
8. Ramachandran Nair, K. G. and Gopakumar, K., *J. Fd Sci. Technol.*, 1977, **14**, 268.
9. *Official Methods of Analysis*, Ass. off. Agric. chem., 12th Ed. 1975.
10. *Manometric Techniques*, Burges Publishing Company, 1959

Anthocyanins in Phalsa (*Grewia subinaequalis* L.) Fruits*

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Based on paper chromatography and spectral characteristics, two anthocyanin pigments viz., delphinidin-3 glucoside and cyanidin-3—glucoside have been identified in the 'Phalsa' fruit.

Phalsa (*Grewia subinaequalis* L.) belongs to the family Tiliaceae. Phalsa fruits possess a very attractive colour, ranging from crimson red to dark purple. The juice, also possesses a deep red colour and has a pleasing flavour. It is considered to have a cooling effect during the hot weather.[1]

The colour of the fruit is due to water soluble anthocyanin pigments, the nature of which has not been reported in the literature. Investigations carried out to identify the same are reported.

Materials and Methods

The ripe fruits (100g) harvested from the experimental orchard of the Institute were used. The peels which contain the pigments were separated from the fruit by hand and blended with methanol containing 0.33 per cent HCl, filtered and washed with the same solvent until the residue was colourless. The combined filtrate was concentrated in a rotary flash evaporator (below 40°C) under vacuum. The concentrate was shaken twice with 200 ml of dry ether when the pigment partially precipitated as a semi-solid mass.

Isolation of pigments: The pigment extract was streaked on 46 × 57 cm sheets of Whatman No. 3 paper and developed by descending chromatography using 1 per cent HCl for 18-24 hr. Two distinct bands designated as P_1 and P_2 separated. The chromatograms were air dried, the band eluted with methanol containing 0.1 per cent HCl, and the solvent evaporated in vacuum below 40°C. Each pigment was further purified using the same solvent.

Identification: The pigments were identified by spectroscopic and chromatographic procedures described by Harborne[2]. Visible spectra of the purified pigments dissolved in 0.1 per cent HCl in methanol were taken using Hitachi-124 spectrophotometer. Shift in absorption maximum was determined by adding 3 drops of 5 per cent $AlCl_3$ in ethanol (w/v) to 3 ml of pigment solution.

R_f in different solvents (Table 1) were determined using Whatman No. 1 paper.

Acid hydrolysis: The isolated pigment was heated with 2 N HCl at 100°C for 30 min, cooled, and the anthocyanidin(s) extracted with iso-amyl alcohol. The aqueous phase was extracted with di-N-octylmethylamine to remove the acid. Both the solutions were concentrated by evaporation. The aglycones were identified by paper chromatography in different solvents using delphinidin and cyanidin obtained from brinjal skin and red rose petals respectively as reference material. The sugars were identified using BAW and BPW, solvents and aniline hydrogen phthalate as spray reagent[4].

Partial acid hydrolysis: The anthocyanin chromatographically separated was dissolved in 2 N HCl and heated in boiling water bath for 5,10,15,30,45 and 60

TABLE 1. SOLVENT SYSTEMS FOR CHROMATOGRAPHY

Composition (V/V) and proportion

1. Glacial acetic acid-water-concentrated hydrochloric acid (15:82:3) (AWH)
2. Concentrated hydrochloric acid-water (3:97) 1% HCl
3. Glacial acetic acid-concentrated hydrochloric acid-water (5:1:5) (HAC-HCL)
4. n-butanol-glacial acetic acid-water (4:1:5) upper phase (BAW)
5. n-butanol-2N HCl (1:1). Upper phase (BU-HCl)
6. n-butanol-pyridine-water (6:4:3) (BPW).
7. Glacial acetic acid-concentrated hydrochloric acid-water (30:3:10) Forestal)
8. Formic acid-concentrated hydrochloric acid-water (5:2:3) (Formic)

*Part of research work carried out by the first author for the award of Ph.D. degree.

TABLE 2. R_f (x100) VALUES AND SPECIAL PROPERTIES OF PHALSA ANTHOCYANIDINS

Pigment	Solvents					'Absorption max (MeoH-HCl)		Al-shift	Identification
	HAC-HCl	BU-HCl	BAW	Forestal	Formic	Visible (nm)	U. V. (nm)		
P_1	22	37	40	27	9	545	275	+	Delphinidin
P_2	33	73	62	43	15	535	275	+	Cyanidin
Delphinidin*	22	38	40	29	8	545	275	+	
Cyanidin*	35	76	62	43	17	535	275	+	

*Authentic aglycone.

min interval and chromatographed using AWH[5]. This test helped to distinguish the type of glycosides. The monoglycoside gave only two spots viz. the monoglycoside and the aglycone.

Hydrogen peroxide oxidation: Sugars attached to the 3 position were identified by the procedure of Chandler and Harper[6]. The pigment isolates were dissolved in methanol (0.2 ml). Forty microlitre of hydrogen peroxide (30 per cent) was added, allowed to stand for 4 hr and treated with few grains of Pd catalyst at room temperature to effect decomposition of excess peroxide. After 20 hr 50 μl of ammonia was added, the solution warmed for 5 min in a boiling water bath, and the resultant solution chromatographed directly for analysis of sugars.

Alkaline hydrolysis: The anthocyanins were analysed for acylation by the method of Philip.[5]

Results and Discussion

The colour was due to two types of anthocyanin pigments viz. P_1 and P_2 whose own colour was purple and pink respectively. Change of P_1 and P_2 colours to blue and violet with addition of aluminium chloride indicated the presence of two free hydroxyl groups in the ring.[2] These pigments failed to show any fluorescence in ultra-violet light indicating that they did not belong to the 3,5-diglycoside types.

The anthocyanidins of the P_1 and P_2 pigments have been identified as delphinidin and cyanidin respectively based on their R_f values in different solvents and spectral properties (Table 2). The R_f and Spectral values and characteristics found were comparable to the values reported by Bate-Smith[7] and Harborne.[3,8]

The sugar moieties released by acid hydrolysis and hydrogen peroxide oxidation from both the pigments consisted of glucose only (Table 3). Both P_1 and P_2 yielded no intermediary product on partial acid hydrolysis which indicated them to consist of only 3-monoglucosides of delphinidin and cyanidin respectively. Cc-chromatography of the ether extract of the alkaline

TABLE 3. R_f (x100) VALUES OF SUGAR MOIETIES IN PHALSA ANTHOCYANINS

Sugar	Solvents		Sugar identified	Colour (Aniline hydrogen phthalate spray)
	BPW	BAW		
P_1	30	17	Glucose	Brown
P_2	30	17	Glucose	Brown
Glucose	30	19		Brown
Arabinose	35	26		Pink brown

Same values and colour were obtained for P_1 and P_2 after H_2O_2 oxidation also.

TABLE 4. R_f (x100) VALUES AND SPECTRAL PROPERTIES OF PHALSA ANTHOCYANINS

Pigment	Solvents					Absorption max (MeoH-HCl) (nm)	Absorption ratio A 440 / A Max (%)	Al shift
	AWH	HAC-HCl	BU-HCl	BAW	1% HCl			
P_1	12	44	12	26	2	540	25	+
P_2	23	57	21	40	5	530	21	+

hydrolysate indicated no acylation. Based on R_f values and spectral properities (Table 4), and those reported in literature[9,10] the anthocyanins present in Phalsa fruit have been identified as delphinidin-3-glucoside and cyanidin-3-glucoside respectively.

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References

1. Kirtikar, K. R. and Basu, B. D., *Indian Medicinal Plants*, Published by Basu, L. M., Allahabad, India, 1933, Vol. I
2. Harborne, J. B., *Comparative Biochemistry of the Flavonoids*, Academic Press, London, 1967.
3. Harborne, J. B., *J. Chromatog.*, 1958 *b;* **1,** 473.
4. Partridge, S. M., *Nature, Lond.*, 1949, **164,** 443.
5. Philip, T., *J. Fd Sci.*, 1974, **39,** 449.
6. Chandler, B. V. and Harper, K. A., *Austr. J. Chem.*, 1961, **14,** 586.
7. Bate-Smith, E. C., *Biochem., J.*, 1954, **58,** 123.
8. Harborne, J. B., *Biochem. J.*, 1958, *a*, **70,** 22.
9. Somers, T. C., *J. Sci. Fd Agric.*, 1966, **17,** 215.
10. Chan, H. T., (Jr). Jan-Kao, T.H.C. and Nakayama, T.O.M., *J. Fd Sci.*, 1977, **42,** 19.

RESEARCH NOTES

STUDIES ON PECTOLYTIC AND CELLULOLYTIC ENZYMES IN MANDARIN ORANGES INFECTED BY *ALTERNARIA ALTERNATA* (FR) KEISSL

Activities of pectolytic and celluloytic enzymes increased in Mandarin oranges following infection by *Alternaria alternata* (Fr) Keissl. Polygalacturonase (PG), and polymethylgalacturonase (PMG) activities increased upto 10 days after infection in edible portion and tended to drop thereafter. In rind, however, activities of PG and PMG continued to increase upto 13 days. Pectinmethylesterase (PME) and carboxymethyl cellulase (CMCase) activities also increased after infection but exhibited peaks in early stages of pathogenesis. PG activity appeared to be predominant in rind.

Degradation of cell wall in fruits is believed to be the beginning of the infection process. Pectolytic and cellulolytic enyzmes are the most important in this respect. A fungus isolated from infected Mandarin oranges from Nagpur area was identified as *Alternaria alternata* (Fr) Keissl which had been found to cause soft rot in oranges.[1] According to a recent report[2], this organism has not been so far reported on Mandarin oranges. In view of the importance of the pectolytic and celluloytic enzymes in the infection process and also the possibility that different types of infections might lead to varying qualitative and quantitative alterations in profiles of these enzymes, experiments were conducted to study the pattern of changes in activities of pectolytic and cellulolytic enzymes in Mandarin oranges infected by *A. alternata* (Fr) Keissl.

Healthy oranges of uniform size and maturity were procured from "Telankhedi orchard", Punjabrao Agricultural University, Nagpur. These were artificially inoculated with spores of *Alternaria alternata* (Fr) Keissl. Fruits were analysed for various enzyme activities at predetermined intervals upto 13 days after inoculation. Normal, uninoculated fruits were also analysed. Enzyme extracts were prepared according to the procedure of Riov.[3] Polygalacturonase (PG), and polymethylgalacturonase (PMG) activities were assayed by the method of Riov[3]. Pectinmethylesterase (PME) activity was assayed by the procedure of Hancock *et al.*[4] and the method of Trager[5] was employed for the assay of carboxymethyl cellulase (CMCase). One unit of PG activity and PMG activity is that amount of the enzyme which catalyses liberation of 1 μ mole of galacturonic acid in 24 hr. One unit of PME activity is that amount of the enzyme which liberates 1 μ equivalent of carboxyl groups in 30 min. One unit of CMCase activity is the amount of the enzyme which liberates 1 mg of glucose in 24 hr.

Several early studies failed to detect PG activity in oranges, Mandarins and lemons; it was suggested that the citrus tissue possibly contains a PG inhibitor. Riov[6] pointed out that this apparent absence of PG activity in some systems is due to an enzyme, uronic acid oxidase, which oxidises the reaction products of PG as well as the free reducing groups of the substrate. Use of sodium hydrosulphite as an inhibitor of uronic acid oxidase was therefore, recommended during the assay of PG. We have observed that addition of sodium hydrosulphite does not influence the PG or PMG activity in Mandarin oranges (data not presented).

Table 1 shows the activities of pectolytic and cellulolytic enzymes following infection by *A. alternata* (Fr) Keissl. All enzymes showed increase following the infection but the degree of such an increase was different for different enzymes. PME activity reached a maximum on the 3rd day both in the rind and in edible portion and then decreased sharply. PG and PMG activities continued to increase upto 10 days in edible portion and upto 13 days in rind. *Alternaria* rot is a centre rot and probably that is the reason for the early maximum production of PG and PMG in edible portion. CMCase activity was found to be maximum after 3 days of infection, both in rind and edible portion. In control fruits, activities of all the enzymes tended go up to some extent during major period of the experiment after which they tended to drop. It was also seen that, after infection, production of PG in rind exceeded production of PMG. Not much difference in this respect could be observed in edible portion.

Hussain and Kelman[7] and Friedman[8] tried to find a causal relationship between pectolytic activity and pathogenesis. They could not assign a precise role to PME but PG levels were found to be increased during infection. Friedman[8] further suggested that exposure of pectins between primary and secondary cell walls to pectin degarding enzymes is possibly dependent on prior cellulolytic activity. It thus appears possible that in *Alternaria* rot, CMCase formed in early stages of pathogenesis exposes the pectins which are then demethylated by PME to pectic acids. Pectic acids thus formed probably contribute to the stimulation of PG and PMG production. *In vitro* studies carried out by the authors have indicated that *A. alternaria* (Fr) Keissl is not

TABLE 1. PECTOLYTIC AND CELLULOLYTIC ACTIVITIES IN MANDARIN ORANGES DURING STAGES OF PATHOGENESIS DUE TO *ALTERNARIA ALTERNATA* (FR) KEISSL

Enzymes	Infection period (days)	Enzyme activity (units/g fresh wt.)			
		Control fruits		Infected fruits	
		Rind	Edible part	Rind	Edible part
PME	0	80.0	44.4	85.3	49.7
	3	76.3	50.6	111.9	89.7
	6	85.7	43.4	24.8	20.6
	10	—	—	24.8	19.8
	13	62.5	32.0	22.2	15.4
PG	0	41.8	19.0	47.5	19.0
	3	45.3	17.8	102.6	66.5
	6	50.5	24.5	141.1	104.5
	10	—	—	456.0	408.5
	13	20.3	11.6	560.5	304.0
PMG	0	47.5	28.5	47.5	38.0
	3	48.7	39.2	57.0	95.0
	6	56.4	43.8	76.0	138.7
	10	—	—	96.9	368.6
	13	32.1	20.4	152.2	76.0
CMCase	0	120.0	40.0	120.0	60.0
	3	124.5	61.1	400.8	166.6
	6	139.2	69.5	168.6	124.5
	10	—	—	165.4	108.0
	13	80.3	34.7	83.2	22.3

PME, Pectin methylesterase; PG, Polygalacturonase,; PMG, Polymethylgalacturonase; CMCase, Carboxymethyl cellulase.

capable of extracellular production of PME but produces PG and PMG[9]. It is thus possible that PME activity found in fruit tissues is that of host origin and significant amounts of PG and PMG are contributed by the pathogen.

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References

1. Kunte, S. and Shastri, N. V., *Sci. Cult.*, 1979, **45**, 489.
2. Bilgrami, K. S., Jamaluddin and Rizwi, M. A., *Fungi in India, Part I. List and References*, Today and Tommorow's Printers and Publishers, New Delhi, 1979.
3. Riov, J., *J. Food Sci.*, 1975, **40**, 201.
4. Hancocok, J. G., Mirror, R. L. and Lorbeer, J. W., *Phytopathol.*, 1964, **54**, 928.
5. Trager, W. W., *Biochem. J.*, 1932, **26**, 1762.
6. Riov, J., *Plant Physiol.*, 1974, **53**, 312.
7. Hussain, A. and Kelman, A., *Phytopathol.*, 1958, **48**, 377.
8. Friedman, B. A. and Ceponis, M. J., *Phytopathol.*, 1959, **49**, 277.
9. Kunte, S. and Shastri, N. V., *Indian J. Microbiol.* (in press).

QUANTITY AND QUALITY OF PECTIN IN SUNFLOWER AT VARIOUS STAGES OF MATURITY

The pectin content of sunflower is found to increase from the stage of full blossom to the complete matured dried flower heads, but as the sunflower grows and matures, the jelly grade of pectin goes on decreasing.

Sunflower (*Helianthus annus* L.) is widely grown in our country as an oilseed crop. Mature sunflower without seeds is a waste but is a good source of pectin. According to the reports of various investigators[1–5], the flower heads contain 14 to 26 per cent pectin on dry weight basis. The object of this investigation was to find out variations in the quantity and quality of pectin present in sunflowers at various stages of maturity.

The sunflower variety 'Romsun Record', grown at Oilseeds Research Station (C.S.A. University of Agriculture and Technology), Kanpur, was used. The sunflowers were plucked after 100 (prior to seed formation), 130 (seed formation), 160 (seed maturation) and 180 (harvest) days from the date of sowing. Size (diameter) of each sunflower head was measured. From the heads of the green sunflowers of the latter three stages, the seeds were removed and then the heads cut into 0.5 in pieces. These pieces were dried at 50°C in a still air oven. The dried samples were powdered in a laboratory mill and used for extraction of pectin. The procedure[6], mentioned below, was adopted for the extraction and recovery of pectin from the samples at all stages.

A sample (10 g) was first soaked in sufficient quantity (100 ml) of ethanol (95 per cent) for 15 min. The mixture was filtered and the residue washed with ethanol-diethyl ether (1:1) mixture, and subsequently with ether alone. The treated sample was suspended in 300 ml solution of oxalic acid and ammonium oxalate (0.5 per cent each) and the mixture was heated to 90°C in a water bath for 30 min with occasional stirring. It was then cooled, filtered, and the insoluble residue was treated again with 200 ml solution of oxalic acid and ammonium oxalate in the same manner. Finally, the residue was washed with hot water until it was free from pectin, and the

TABLE 1. QUANTITY AND QUALITY OF PECTIN OF SUNFLOWERS AT VARIOUS STAGES OF MATURITY

Sampling period (days)	Diam. of flower (in)	Wt. of flower with seed (g)	Wt. of flower without seeds (g)	Flower Moisture (%)	Yield (%) pectin (dwb)	Moisture in pectin (%)	Ash (%)	Free acid (meq NaOH/ 500 mg pectin)	Methoxyl content (%)	Purity AUA (%)	Grade
100	6.25	390	—	87.8	16.6	6.27	3.60	0.4	10.30	89.00	160
	6.25	370	—								
	5.75	343	—								
	5.75	297	—								
	5.00	221	—								
130	6.25	320	237	83.0	20.6	6.38	5.00	1.1	8.52	86.24	140
	5.50	199	116								
	5.25	168	99								
	5.00	142	93								
	4.00	101	64								
160	6.00	121	44	24.0	24.6	6.32	5.90	1.3	7.76	83.60	100
	5.10	93	26								
	4.80	76	24								
	4.80	62	17								
	3.90	36	13								
180	6.00	119	40	13.0	24.3	6.15	5.98	1.3	6.73	78.32	50
	5.00	92	24								
	4.90	73	19								
	4.80	59	15								
	3.70	30	12								

washings were included in the extract. The pectin of the extract was precipitated by acidified ethanol, the precipitate was washed by natural ethanol, and subsequently by diethyl ether. The precipitate of pectin was made solvent-free by rubbing in a large porcelain mortar and dried at 50°C under vacuum. Then, the pectin was stored in a desiccator over night and weighed. Moisture was determined by heating 1 g ground pectin (80 mesh particles) under vacuum (5-20 mm Hg) at 100°C for 4 hr. Ash content was determined by igniting 1 g pectin slowly and then heated at 600°C for 4 hr. Free acidity of pectin was determined by titrating 0.5 per cent pectin solution with standard 0.1N sodium hydroxide solution using phenolphthalein as indicator. Saponification method[7] was used for the determination of methoxyl content and anhydrouronic acid (AUA). Finally the samples of the pectin were tested for jelly grade by Delaware Jelly Strength Tester[8]. The results are given in Table 1.

The pectin content of sunflower increased to maximum level in 160 days, and thereafter remained constant. The ash contents and free acidity of pectin samples were found in increasing order, while the methoxyl contents and anhydrouronic acid contents of pectins were found in decreasing order with maturity of sunflowers. Jelly grade of pectin extracted after 100 days of sowing was found to be maximum but decreased thereafter. Therefore, it is concluded that one of the reasons for lowering of jelly grade of pectin could be due to demethylation of pectin during the maturity of sunflowers.

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References

1. St. Stoikoff., *Mitt. Geb. Lebensmittee unters, u. Hyg.*, 1948, **39**, 292.
2. Shewfelt, A. L. and Worthington, O. J., *Food Technol., Champaign*, 1953, **7**, 336.
3. Bishop, C. T., *Can. J. Chem.*, 1955, **33**, 1521.
4. Riaz, A. and Uddin, M., *Pak. J. sci. ind. Res.*, 1972, **15**, 167.
5. Lin, M. J. Y., Sosulski, F. W., Humbert, E. S. and Downey, R. K., *Can. J. Plant Sci.*, 1975, **55**, 507.
6. Pathak, D. K., *Studies of nature and properties of sunflower pectin*, Ph.D. Thesis, Kanpur University, 1976.
7. Joslyn, M. A., *Methods in food Analysis*, Academic Press, N. Y. and London, 1970, 565-599.
8. Kertesz, Z. I., *The Pectic Substances*, Interscience Publishers, New, York, 1951.

A STUDY OF THE CHANGES UNDERGONE BY TEA POLYPHENOLS IN SOLUTION USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

The changes undergone by the tea polyphenols during storage have been investigated using high performance liquid chromatography. It was found that the principal changes were related to the formation of a group of compounds allied to the thearubigins. There was no marked change in the theaflavin content.

Tea extracts deteriorate rapidly on storage and turn into a dull dark brown colour, developing an undesireable taste commonly described as "flat". The prevention of such degradative changes is of particular importance in the preparation of stabilised liquid tea preparations such as canned and iced teas. This investigation was carried out to determine the extent to which this deterioration in taste could be associated with chemical transformations in the polyphenolic constituents of the beverage. Changes occurring during the storage of black tea have already been reviewed[1].

The solutions were analysed using a modification of the HPLC procedure described by Hoefler and Coggon[2]. The sample elution system consisted of a linear gradient of (A) 10 per cent acetone, 0.5 per cent acetic acid and 89.5 per cent water and (B) 40 per cent acetone, 0.5 per cent acetic acid and 59.5 per cent water. The eluate was scanned at 380 nm at which wavelength the unoxidised polyphenols did not exhibit any absorbance and only the oxidised polyphenols were detected.

The tea extracts were stored in the dark after initial sampling. At the end of 24 hr storage at room temperature it was found that the astringent taste was completely lost. The principal change in the chromatogram was a very marked decrease in the theaflavin gallates with corresponding increase in the theaflavins due to contamination of the extract by *Aspergillus*. This contamination was prevented in subsequent experiment by using 0.1 per cent sodium benzoate or preferably benzoic acid as a preservative.

TABLE 1. THE QUALITY ASSESSMENT OF TEA EXTRACTS DURING STORAGE

Storage condition	Period of storage (h)			
	24 hr	48 hr	72 hr	168 hr
Room temp (24-28°C)	Satisfactory	Satisfactory	Poor	Poor
Aerated at room temp (oxygen at 30 ml/min)	Satisfactory	Poor	V.poor	V.poor
Stored at 55-60°C	Poor	V.poor	V.poor	V.poor

There was a slower but progressive loss of tea charac ter even in the presence of a preservative. The purpose of this investigation was to characterise these changes. Experiments were carried out using three storage conditions as detailed in Table 1, to investigate the effects of oxygen and temperature on these degradative changes. Experiments were carried out for 7 days and the stored solutions were sampled every 24 hr for 3 days and on the 7th day. The assessment of quality based on the losses of liquor colour and taste is shown in Table 1. It was evident that quality deterioration was most rapid at elevated temperatures when the liquid turned dark brown in 24 hr. The loss in quality was very noticeable after 48 hr in (2) and 72 hr in (1).

HPLC analysis was carried out every 24 hr on all the samples. It was found that the principal change occurring on storage which related to a loss of quality was the appearance of a new group of peaks on the chromatogram in the region associated with the thearubigins. This is illustrated in a typical chromatogram of the sample maintained at an elevated temperature, after 168 hr storage (Fig. 2). It is very significant that there is no noticeable loss in the theaflavin content evident when this chromatogram is compared to Fig. 1. The increase in the thearubigin fraction is accelerated primarily by heat and somewhat by aeration.

It is possible to conclude that the development of the dark colour and 'off taste' in tea liquor on storage is

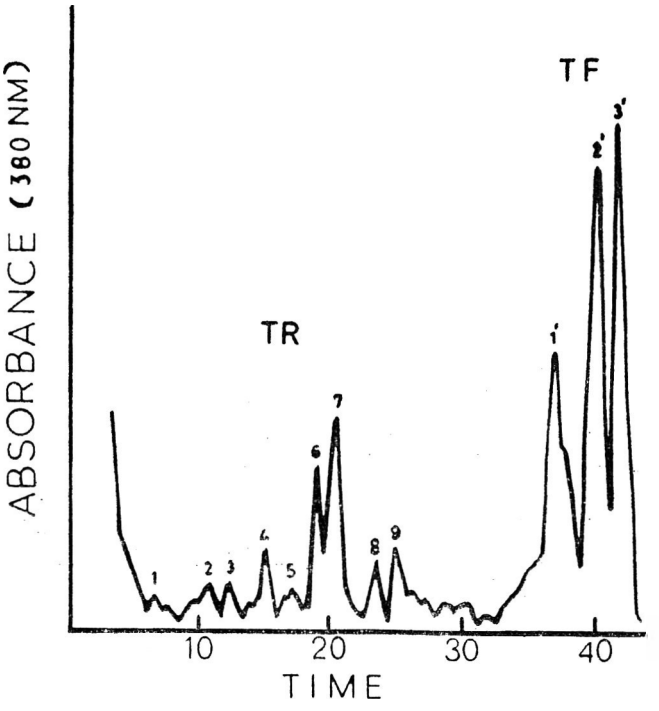

Fig. 1. HPLC chromatogram of a fresh tea extract TF, Theaflavins; TR, Thearubigins

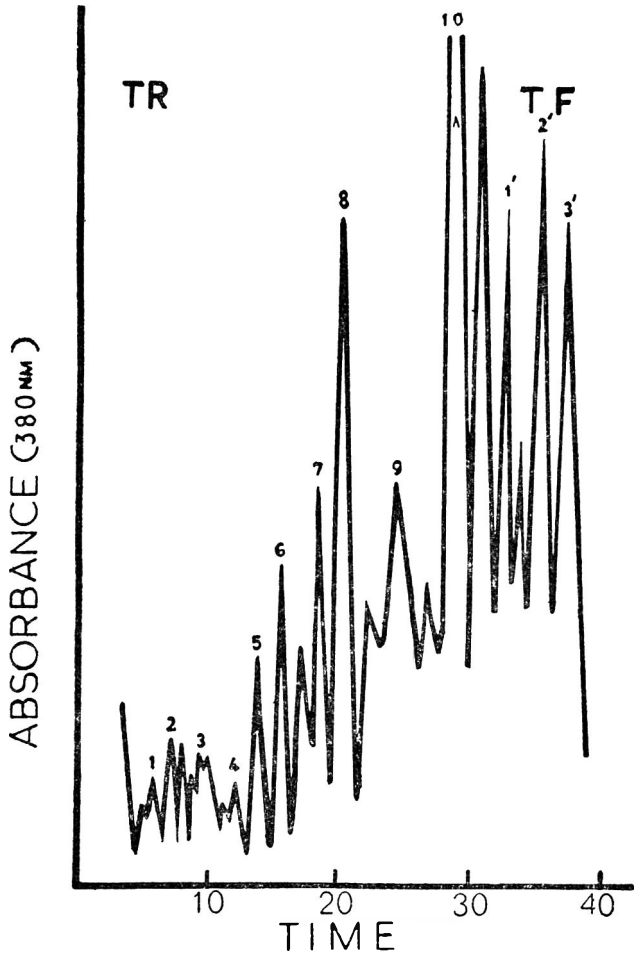

Fig. 2. HPLC chromatogram of a tea extract stored for 168 hr at an elevated temperature.

TF, Theaflavins; TR, Thearubigins.

not related to a loss of theaflavins but rather due, at least partially, to the formation of oxidised and polymerised polyphenolic derivatives (thearubigin type), from the unoxidised and partially oxidised compounds which contribute significantly to the brisk taste of the beverage[3].

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References

1. Stagg, C. V., *J. Sci. Fd Agric.*, 1974, **25**, 1015.
2. Hoefler, A. C. and Coggon, P., *J. Chromatog.*, 1976, **129**, 460.
3. Sanderson, G. W., Randive, A. S., Eisenberg, L. S., Farrel, F. J., Simons, R., Manley, C. H. and Coggon, P., *A.C.S. Symposium Series No.* 26. Ed. Charalambous, G. and Katz, I. Amer Chem Soc. 1976.

A NOTE ON THE EFFECT OF FREEZING GRAPE BUNCHES ON THE COMPOSITION OF MUSTS AND WINES*

The effect of freezing grapes on the composition of musts and wines was investigated using five varieties. It was observed that with most of the varieties the musts and wines from frozen (−10°C) grapes had slightly lower acidity and higher pH as compared to control. In general, the wines made from frozen grapes had lower tannin content and better colour.

Generally, wine is made from fresh grapes. Though wine is made from grape juice concentrate, the quality of such wine is not comparable to the wine made from fresh grapes. It is reported[1] that wines made from grapes which are frozen rapidly and thawed immediately develop undesirable odour, flavour and colour. Brown[2] reported that addition of antioxidant to crushed grapes and storing under frozen condition prevented the development of undesirable odour, flavour and colour in wines. However, the results presented in this note indicate that freezing of grapes at −10°C for one month did not affect the quality of wines to a great extent.

Five grape varieties namely, 'Anab-e-Shahi', 'Excelsior', 'Thompson Seedless', 'Bangalore Blue' and 'Black Champa' grown at the Experimental Farm of the Indian Institute of Horticultural Research at Hesseraghatta, Bangalore were used. The grapes were separated into two lots; one was crushed and fermented by traditional fermentation and the other was washed thoroughly in running water, drained, packed in polyethylene bags and stored in the freezer at −10°C. After one month, the grape bunches were removed, thawed, crushed and fermented. The sugar content of both control and frozen grapes was adjusted to 22° Brix with cane sugar. The procedure followed for wine making and the methods used for must and wine analysis have already been described[3,4].

Freezing of grapes did not alter the sugar content of the musts of all the varieties (Table 1). But, there was a slight decrease in the acidity of musts of all the varieties, except in 'Bangalore Blue'. The reduction in acidity resulted in the increase of pH of musts. After one month storage at freezer, the berries were intact without any visual damage. However, there was a slight browning in white varieties.

The wines made from frozen grapes showed a slight decrease in acidity ranging from 0.01 to 0.13 per cent except in 'Anab-e-Shahi'. The change in the pH of wines was very marginal. The significant observation was with regard to tannin content and colour of wines. The

*Contribution No. 1002

TABLE 1. EFFECT OF FREEZING GRAPES ON THE COMPOSITION OF MUSTS AND WINES

Variety	Treatment	Must Composition			Wine Composition					
		T.S.S. (°Brix)	Acidity (as g tartaric acid/100 ml)	pH	Acidity (as g tartaric acid/100 ml)	pH	Tannin (mg/l)	Colour (O.D. at)* 420 nm	520 nm	420/520
Anab-e-shahi	Frozen	14.8	0.53	3.65	0.58	3.70	688	0.168	—	—
	Control	15.0	0.57	3.60	0.53	3.60	856	0.301	—	—
Excelsior	Frozen	19.4	0.92	3.60	0.70	3.80	742	0.301	—	—
	Control	19.2	0.95	3.50	0.77	3.70	748	0.387	—	—
Thompson seedless	Frozen	19.8	0.77	3.55	0.65	3.50	636	0.276	—	—
	Control	19.2	0.81	3.45	0.66	3.40	728	0.252	—	—
Bangalore blue	Frozen	17.6	1.05	3.20	0.93	3.25	1140	1.610	1.370	1.18
	Control	17.4	1.00	3.20	1.06	3.30	1320	1.250	1.250	1.00
Black champa	Frozen	18.0	0.75	3.60	0.70	3.75	1320	1.140	0.860	1.33
	Control	18.0	0.83	3.40	0.71	3.75	1880	1.310	0.970	1.35

*White wines were measured undiluted, while red wines were measured with 1:10 dilution.

wines made from frozen grapes had lower tannin content than control wines. Though it is known[4] that heating grape musts increased the tannin content, no information is available on the effect of freezing grape on tannin content of wine prepared from it. However, storage of wines at low temperature reduced the tannin content[5]. Among the three white varieties tested, the colour of wines made from frozen 'Anab-e-Shahi' and 'Excelsior' were better than the wines made from control grapes. There was not much change in case of wine from 'Thompson Seedless'. With regard to red varieties, the colour of control wine from 'Bangalore Blue' and the wine from frozen grapes of 'Black Champa' was better. These preliminary observations suggest that probably there is a varietal response to frozen storage. This investigation indicated that freezing grape bunches did not alter the quality of wines to a great extent and frozen grapes could be used for wine making in off-season. Flora[6] also observed that freezing did not affect the flavour and colour of juice of grapes that had been held at −28°C for 9 months.

The authors are thankful to Drs. G. S. Randhawa and K. L. Chadha for their interest in this work and for providing the necessary facilities. They are also thankful to Drs. Rajendra Singh and H. C. Dass for providing the grape material used in this investigation.

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References

1. Sayre, R. N., Brown, M. S., Finkle, B. J. and Pereira, E.S.B., Proc. 13th Intern. Cong. Refr. Washington, D. C., Vol. 3, 1971.
2. Brown, M. S., *Am. J. Enol. Vitic.*, 1975, **26**, 103.
3. Ethiraj, S. and Suresh, E. R., *J. Fd Sci. Technol.*, 1978, **15**, 111.
4. Ethiraj, S. and Suresh, E. R., *J. Fd Sci. Technol.*, 1978, **15**, 195.
5. Singleton, V. L. and Esau, P., *Phenolic substances in grapes and wines, and their significance*, Academic Press, New York, London, 1969, 175.
6. Flora, L. F., *Am. J. Enol. Vitic.*, 1976, **27**, 84.

SOIL-SALT MIXTURE FOR PRESERVING WET PADDY

Problems exist in storing and handling of common salt treated wet paddy. To avoid this, wet paddy was treated with salt incorporated soil or soil-sand mixture for extracting and holding the water *in situ*. A salt-soil (or soil to sand in equal proporation) mixture when mixed with wet paddy in the ratio of 0.75:1 and an optimum level of 5% common salt by the weight of paddy effectively preserved the wet paddy without fungal infection or development of heat for 10 days.

Paddy harvested in rainy season cannot be dried in sun and it spoils rapidly. Mixing of various chemicals[1–5] were tried earlier to prevent the spoilage. Of these, common salt (NaCl)[1,3] preserved the grain by withdrawing moisture from it; but if the extracted water is not removed from the surface, the paddy gets heated and infected. To overcome this problem, different absor-

bents[3,6] were used. Earth, saw dust and paddy husk when mixed repeatedly with wet paddy extracted moisture and preserved it[7]. Considering the cost and labour involved in the above treatments, a cheap method of using soil-salt mixture for extracting moisture from paddy has been tried.

Natural saline soil (available in coastal area containing 5.5 per cent NaCl) or soil and salt mixture (sieved through 20-mesh sieve) was mixed with wet paddy and either filled in jute bag or heaped over a polythene sheet. The soil-salt mixture was prepared by dissolving the required quantity of salt in 12.5 l of water so as to give, 2, 3, 4 and 5 per cent of salt on the weight of paddy when it was added to paddy in the prescribed proportion. This salt solution was added to 100 kg of soil or a mixture of soil and sand followed by drying in sun. This mixture was added to wet paddy (50 kg lots) in 0.25:1, 0.50:1, 0.75:1 and 1:1 proportions. Not much difference was observed in the absorption of moisture in paddy stored in the bag or heap. Hence the results obtained with the bag storage alone are reported. Observations were recorded for sprouting, infection, heating and change in the colour of paddy during storage. The grain moisture was determined after shelling in a Satake grain testing mill (without operating the blower). The loss in dry weight of grain was determined in 1 kg sample as described[4]. Moisture content of the soil-salt mixture was determined in 5-g sample and the salt (NaCl) content after extracting the salt in warm water[8].

River sand or sea sand or soil alone without salt when mixed with wet paddy did not control sprouting, infection and heating (Table 1); but coastal saline soil or prepared saline soil, preserved wet paddy up to 2 months. In two days' time, the kernel moisture dropped from 27.0 to 16.5 per cent when the mixture to paddy was in equal proportion when the salt content was 5 per cent by weight of paddy, after which the lowering of moisture was rather slow-a decrease of 1.5 per cent was observed in 13 days. In the dry salt also, a similar pattern of lowering of moisture was observed. The above soil-salt mixture containing 0.5 per cent initial moisture when mixed extracted 10.3 percent moisture in 10 days' storage with the paddy in the same manner and the extracted water did not ooze out of the mixture; thus it avoided the wetting of the container or spoiling of the godown floor. A minimum salt of 5 per cent by weight of paddy was required as in the case of dry salt treatment[1,3] for proper extraction of moisture and to prevent infection (Table 2).

Sand from the river or sea containing 5 per cent salt (by weight of paddy) when mixed with wet paddy containing 25.8 per cent moisture reduced the moisture of paddy to 18 per cent only; the water which was extracted by sand oozed out of the jute bag and the grains got slightly infected in a week. Similarly when the soil in the mixture was replaced by sand to less than 50 per cent, it did not effectively preserve the wet paddy. In 5 days' time the paddy temperature (Co 25 variety with 25 per cent initial moisture; R.H. 80-90 per cent) was 38°C and by 9 days it went up to 53°C. This indicated that the moisture was not properly extracted from the paddy. When the proportion of soil-salt mixture to paddy was less than 0.75:1, the grains got damaged by heating

TABLE 2. CONDITION OF PADDY 72 HOURS AFTER TREATING WITH SOIL-SALT-MIXTURE

Salt in soil* (%)	Kernel moisture (%)	Infection
2	18.6	Heavy
3	18.0	Heavy
4	17.4	Stray
5	16.5	Nil

*Adjusted to the wt of paddy treated
Variety 'Co 25'; Initial moisture content 25.3%

TABLE 1. MOISTURE AND CONDITION OF PADDY DURING SOIL TREATMENT

Medium*	Storage days (No.)	Kernel moisture (%)	Temp. (°C)	Germination	Infection
Sea sand	4	18.5	34	Incipient	Heavy
Soil	4	20.5	37	,,	Heavy
Soil-salt mixture	15	15.0	30	Nil	Nil

*Contained 5% salt on the weight of paddy and mixed with paddy at 1:1 ratio
Variety 'Co 25'; Initial moisture content 25.8%

TABLE 3. CONDITION OF PADDY SEVEN DAYS AFTER MIXING WITH DIFFERENT PROPORTIONS OF SOIL-SALT MIXTURE

Soil-salt mixture to paddy ratio (by wt)	Kernel moisture (%)	Paddy temp. (°C)	Infection
1:1	13.7	28	Nil
0.75:1	15.3	28	Nil
0.50:1	17.3	50	Heavy
0.25:1	20.2	50	Heavy

Containing 5% common salt to the paddy
Variety 'Co 25'; Initial moisture content 24.0%

TABLE 4. YIELD OF BROWN RICE (% DRY BASIS) DURING STORAGE OF WET PADDY TREATED WITH AND WITHOUT SOIL-SALT MIXTURE

Storage period (days)	Co 25		IR 20	
	Control	Treated	Control	Treated
0	77.7	77.7	75.9	75.9
3	76.7	77.5	74.4	75.6
6	76.0	77.3	—	—
9	74.9	77.0	—	—
12	—	—	71.5	74.7
15	—	—	69.7	74.7
20	72.0	77.0	69.3	74.7

Initial moisture content of 'Co 25' was 25.5% and IR-20 was 2.3%;

Soil-salt mixture treated with paddy at 1:1 proportion, salt being 5% on the wt. of paddy.

(Table 3). While using roasted earth for extracting moisture from wet paddy an earth to paddy ratio in the range of 0.50:1 to 0.75:1 appeared to be optimum[7].

Whenever soil alone mixed with salt was used for extracting water, its complete separation from paddy by sieving was difficult; but the separation was easier when the mixture contained equal proportion of sand and soil. The residual sodium chloride in paddy was 0.8 per cent and in brown rice 0.3 per cent. The sieved paddy was washed thrice with water and this on par-boiling yielded excellent rice with practically no breakage. Besides arresting sprouting and development of heat, the treatment of wet paddy with soil-salt mixtur minimised the dry matter loss considerably (Table 4).

It was observed by Srinivas *et al.*[7] that mixing soil containing 1.0 per cent acetic acid or 3.0 per cent burnt lime or roasting the soil to 150°C followed by mixing with wet paddy immediately and keeping in jute bags did arrest the infection; but repeated mixing (4 times) of roasted soil with wet paddy and holding for 2 hr after each mixing reduced the moisture content to a safe level.

Sincere thanks are due to late Dr. V. Subrahmanyan for his guidance and constant encouragement during the course of this study.

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References

1. Shivanna, C. S. and Kudharathulla, B., *J. Fd Sci. Technol.*, 1971, **9**, 129.
2. Shivanna, C. S. and Kudarathullah, B., *Bull. Grain Technol.*, 1971, **9**, 269.
3. Vasan, B. S., Iengar, N.G.C., Nagasubrahamanian, M., Bhasker, R., Shivanna, C. S., Mohandoss, R. and Subrahmanyan, V., *Proc. Indian Nat. Sci. Acad.*, 1973, **39B**, 32.
4. Pillaiyar, P. and Vasan, B. S., *Malays. agric. J.*, 1980, **52**,
5. Vasan, B. S. and Pillaiyar, P., *Oryza.*, 1980 **18**(1) (communicated).
6. Anthoni Raj, S., Singaravadivel, K., Vasan, B. S. and Venkatesan, V., *Indian J. agric. Sci.*, 1980, **50**, 675.
7. Srinivas, T., Raghavendra Rao, S. N., Bhashyam, M. K. and Desikachar, H.S.R., *J. Fd Sci. Technol.*, 1976, **13**, 142.
8. *Official Methods of Analysis*, Assn. Official Analytical, Chemists, Washington Ed. XI, 1970, 566.

ERRATUM

Effect of Incorporation of Starch phosphate on the quality of bread by K. Leelavathi et.al, in this Journal 1981, **18** (1), page 6 Table 5 column.

The title of the column shoud be read as "Loaf Volume" instead of "Local Volume".

BOOK REVIEWS

Coffee Technology[3] by M. Sivetz and N. W. Deroisier; The Avi Publishing Company Inc., Westport, Connecticut, 1979, pp 716.

This is a revised edition of the earlier compilation entitled Coffee Processing Technology (1963). The earlier publication consisted of 2 volumes which have been combined into one.

The present edition is a slightly condensed version compared to the earlier two volumes. This has been accomplished through the omission of descriptive details of machinery and equipment employed in the coffee industry.

The 17 chapters of the present edition in 5 parts cover *i*) history of coffee; *ii*) green coffee technology; *iii*) roast coffee technology; *iv*) instant coffee technology; and *v*) coffee and its influence on consumers.

Part I of the current edition on 'history of coffee' corresponds to the first chapter with the same title in the earlier edition. However, it has been split into 3 chapters on the development of coffee plantations, coffee industry and coffee uses. The changing trends in world production, consumption and trade have been discussed in greater detail as also the coffee drinking pattern and habits of the population in different regions of the world.

Part II of the present edition on green coffee technology is divided into 4 chapters on coffee horticulture; harvesting and handling green coffee beans; drying green beans; and hulling, classifying, storing and grading green coffee beans. The 3 separate chapters on sundrying of coffee, machine drying of coffee: drying principles and machine drying of coffee: description of driers in the earlier edition have been combined into a single chapter on drying green coffee beans. A noteworthy addition is on the Shivvers drier and the fluidized bed drier.

Part III on roast coffee technology is split into 2 chapters on coffee bean processing and packaging of roasted ground coffee. In the former chapter, the effect of various roasting parameters on aroma development during roasting is dealt with in greater detail. The inclusion of a separate chapter on packaging roasted ground coffee beans bears testimony to the growing importance of this aspect in the present day context.

Part IV on instant coffee technology consists of the following 4 chapters: percolation: theory and practice; spray drying and agglomeration of instant coffee; aromatizing souble coffees; and freeze dried coffee production. In this part, various aspects on instant coffee-such as effect of different processing variables on product quality and soluble coffee plant design-which lie widely scattered in the earlier edition, have been brought together under a single chapter.

Part V which is the concluding part comprises chapters on physical and chemical aspects of coffee; physiological effect of coffee and cafferine; caffeine and decaffeination; and brewing technology. The treatment of decaffeination in a separate chapter is in recognition of the growing need for providing low-caffeine coffees to cater to an expanding segment of the population. The new aspect covered in the last chapter pertains to the recent innovation of brewing coffee with paper filters which is rapidly coming into vogue.

In the present edition. bibliography has been provided at the end of only 6 of the 17 chapters; however, this has been brought up to date with emphasis on the more recent publications. Likewise, while the earlier edition gives extensive lists of patents (together with an alphabetical index of inventors in most cases) on such diverse topics as mucilage removal, roasting, percolation, spraydrying, packaging, aromatizing and decaffeination at the end of the relevant chapter, the present edition confines itself to the mention of some new patents at the appropriate places in the body of the text itself.

The general get up of the publication is excellent with copious illustrations depicting the gamut of the steps involved in bringing both regular and instant coffee from the growing plantations to the consumer. Greater care in avoiding some printing mistakes would have enhanced its value. To give just one example, the title of chapter 16 in the contents at the beginning (page vii) is given as brewing technology whereas it should actually read as Caffeine and decaffeination.

The authors deserve congratulations for a significant achievement in assembling a vast amount of coffee processing knowledge which lie widely scattered in the literature. The book would undoubtedly be read with interest and profit by all those associated with this important phase of the food industry.

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A simple method of collecting and drying papaya (pawpaw) latex to produce crude papain: by D.J. Moore, Rural Technology Guide 8 of Tropical Products Institute, 56/62 Gray's Inn Road, London—WCIX 8LU, 1980; PP, 20.

The Tropical Products Institute, London, has brought out a series of guides for rural technology to provide working details of devices suitable for use in rural communities. The guides are fully illustrated and written in simple language. This guide describes a simple method for collecting and drying papaya latex to produce crude papain of good quality. The gadgets/equipment employed for drying are so simple that production units can be established in rural areas with least expenditure. In the first chapter the equipment required for tapping of papayas, latex collection trays made of bamboo rings and canvas or P.E. cloth and latex collecting boxes made of wood, are fully described with suitable diagrams, which can easily be made in rural areas. Then the method of collection of latex, the day and time of collection are described. In the second chapter the designs for building a drying shed and fabrication of hot air drier for drying the latex are given. Full drawings of the furnace made out of brick construction are given to enable one to fabricate the same with least difficulty. Finally, the operation of the oven, methods of drying of latex and packing of dried papain are described. This guide will be very useful for extension workers, other field staff in rural development and to the entrepreneurs desirous of starting this industry.

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The use of Plants and Minerals as Traditional Protectants of stored products: by P. Golob and D. J. Webley. Rep. Trop. Prod. Inst. G. 138 VI; pp 32; Price £ 3.20

This booklet describes the use of naturally occurring materials to protect agricultural produce in storage from insect infestation. The report is in part a summary of replies sent by correspondents in many countries in response to the questionnaire and in part a review of published work.

There are six sections in which more than 160 naturally occurring materials are listed along with the country of use, the source of information and a brief description of the use. Some of the information relates to traditional use and some to the application of materials in laboratory or field trials.

Section 1 consists of 6 tables wherein traditional use of azadirachta, acorus, other plants, leaves, seeds, roots or powders are given. Section 2 comprises of information pertaining to oil extracted from plants which have been tested by topical application to storage pests or field trials. Large scale trials carried out on oils particularly vegetable oils such as groundnut oil, palm oil, citrus and wood oil have been tabulated in section 3. Ashes which are widely used for mixing with food grains is dealt with in section 4. Effectiveness of ash varies considerably due to silica content of the dust and abrasive properties of ashes. Traditional use of sand and minerals are given in section 5 which is divided into silicas including diatomites, clays and lime stones. Last section details miscellaneous materials such as husks, smoke, fire and their use in different countries to keep away insects.

There are 117 references which offer very useful information.

It will serve as a useful reference book, for those involved in infestation control aspects.

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Basic Food Microbiology: by George J. Banwart AVI Publishing Co. Inc. Westport, Connecticut; 1979; pp. 780; Price: $ 22.

After the book on "Microbiology of Foods" by Tanner, a similar book with updated information and with the coverage of newer topics that have been revealed during subsequent years of study was needed. This book under review fills this gap. It contains 13 chapters. The first chapter is of introductory nature. The second chapter is on methodology, of enumeration of the microbes or assessment of their metabolic activity. This is a critical evaluation of different methods including sampling plans.

Third and forth chapters include taxonomical aspects of common organisms associated with food and their metabolism and nutritional needs. Source of different organisms and food as a carrier of illness have been dealt with in fifth and sixth chapters. A treatise on enterotoxins and mycotoxins can be mentioned as a special feature of this chapter. Seventh chapter deals with indicator organisms. This is again a critical appraisal of the existing methods and their significance to the quality assessment of the food. Chapter eight deals with food spoilage in a comprehensive manner including food composition, metabolic activity and degradation of the components. This subject includes fruits and

vegetables, processed foods, meat, fish and poultry products, canned foods, and fats and oils.

The ninth chapter presents the useful aspects of microorganisms in the manufacture of bread, wine, enzymes, etc. Different methods of control of undesirable microorganisims are included in the 10th, 11th and 12th chapters. This chapter gives useful information on chemical preservatives and gaseous and liquid sterilants. Thirteenth chapter gives valuable information on food laws.

This book seems to be unique in its comprehensiveness and in depth coverage of various topics related to food microbiology. This will serve as a useful addition to the academic libraries as well as to the book collections of individual laboratories confined to research and development in the area of food microbiology.

V. SREENIVASA MURTHY
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Mass Culture of Spirulina fusiforms: pp. 60; Price Rs. 50.

The awareness of protein shortage in sixties and seventies had generated great worldwide interest for search of newer protein sources including the one of microbial origin. The production of algal biomass has relevance in this context. Algal systems still receive attention in view of their efficiency of solar energy conversion. The monograph on the culture of *Spirulina fusiformis* from the Research Group of the photosynthesis and energy division of Shri A. M. M. Murugappa Chettiar Research Centre, Madras is to be viewed in this context.

Spirulina probably is the most suited algae for a fresh water cultivation in the Indian context and is amenable for low level technology. The monograph provides useful information on the cultivation and processing of algae. The details of tank construction and nutrient supply to cultures have been dealt well. Data on the potentiality of using this in feeds of fish and cattle though limited, focuses the application aspect of this algae.

The final chapter on a suggested model of algal milk farm which presents algae as a component of an integrated system along with production of fodder grass makes interesting reading.

The monograph is not complete in all aspects of algal technology, nutritional testing or quality control. The cost computations are meagre as it may be difficult to make any prediction based on limited studies on a small scale. The possible problems in production have not also merited attention. Many tables list merely routine data which could have been effectively condensed.

The monograph will certainly strengthen the All India effort in progress of using algae as biofertilizer, biofuel and feed, and food. The effort of the research team headed by Prof. C. V. Seshadri, in developing the *Spirulina fusiformis* mass culturing needs to be commended. The monograph will be very useful to those working in the field of algal technology.

L. V. VENKATARAMAN
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Environmental Health Criteria 15: *Tin and organotin Compounds. A preliminary review:* Health and Biomedical Information Programme, WHO, 1211, Geneva-27, Switzerland; pp 109. Sw. Fr. 7.

The World Health Organisation at Geneva has published a number of monographs in the "Environmental health criteria" series. Experts from various countries in different specialities collect information which is thoroughly discussed at meetings and ultimately a monograph is published. The fifteenth monograph on "Tin and Organotin compounds" is the result of such an effort. The monograph has eight sections dealing with biological effects of tin in man and animals, chemical and analytical methods, metabolic aspects, environmental concentrations and exposures and sources of environmental pollution.

Tin occuring in food and beverages may be derived from tin plate or organotin compounds used as stabilisers for PVC materials. The experts have collated all the information and identified newer areas of work where the research effort should be concentrated. In a single monograph of 109 pages there is a wealth of information both for the novice as well as the expert.

I strongly recommend this WHO monograph to scientists who seek the most up to date information on any aspect of tin and organotin compounds.

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Food Processing Waste Management: by John H. Green and Amihud Kramer (Ed.) AVI Publishing Company, Inc. Westport, Connecticut; pp. 629+xii, U.S. $ 43.

In recent decades growing concern is being experienced about environmental pollution and its consequent danger on humanity and other forms of life. Hence,

waste management has assumed a challenge to scientists, technologists and engineers. The earlier concept of treatment of "end-of-pipe" waste is slowly being replaced by process modification which avoids waste creation.

The authors have brought out all the information in five sections viz., monitoring, in-process modifications, treatment, regulations and economics.

Monitoring specifically deals with initial waste survey, various flow meters and other sampling methods to ensure the quantities and quality of waste.

Various procedures are suggested in in-plant modifications to conserve water-energy and prevent pollutants from entering the waste-water stream. The authors extensively dealt with various house keeping practices to avoid solid waste getting into the waste-water stream. Detailed information on dry caustic peeling technology, methods to reduce the waste and energy consumption during blanching, whey treatment and utilisation, and fishery waste recovery have also been presented in this chapter.

The third chapter on treatment deals with the various treatment systems starting with the conventional municipal treatment system and progressing to latest innovations adapted to food processing situations, biological oxidations, lagoon technology, coagulation and flocculation methods and the collected waste for use in poultry industry and production of single cell protein from food processing wastes. The chapter also covers waste-water treatment in meat packing, poultry processing and rendering industries and various methods adopted for land treatment.

The next chapter gives detailed history of water and other pollution control legislations in the United States and the detailed Federal Laws. These legislations will be helpful in drawing suitable standards by developing countries.

The last chapter deals with important items like economic effects of treating fruit and vegetable processing liquid wastes and various subsidies provided by several government institutions for effecting industrial pollution control.

The book is a welcome addition for libraries and will be very much useful for food scientists, technologists and practising engineers.

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

ASSOCIATION NEWS

Second Indian Convention of Food Scientists and Technologists

Nearly 200 professionals participated in the Second Indian Convention of Food Scientists and Technologists organised by the AFST(I) headquarters in collaboration with CFTRI on February 19-20, 1981 in Mysore. Dr. D. K. Salunkhe, Vice-Chancellor of Mahatma Phule Krishi Vidyapeeth, Rahuri and himself a distinguished food scientist, inagurated the Convention with a stirring assertion that India could feed her millions by using modern systems of agricultural production suited to her needs, bringing cultivable waste lands under cultivation, harnessing water resources to ensure timely supply, and extending food supplies through proper storage, handling, transport and processing. "This is the challenge to us, food scientists and technologists", he said, "to make our land once again *sujalam suphalam* (blessed with plenty of water and food)". In his address of welcome, the Director of CFTRI, Shri C. P. Natarajan, traced the symbiotic relationship that had existed from the very start between the Institute and AFST(I), which had been a source of satisfaction to both. Dr. K. T. Achaya, AFST(I) President, outlined the strength of the Association as enshrined in its recently adopted constitution, and announced that the first international AFST conference would be held in Bangalore on 24-28 May 1982. Dr. K. R. Sreekantiah, Secretary AFST(I) proposed a vote of thanks.

Three symposia were featured during the convention. At each of these, four invited speakers reviewed various facets of the symposium topic, followed by considerable discussion. The titles of the symposia, the speakers and their subjects are shown in the tabular statement. One afternoon was devoted to research papers in the form of poster presentations. Some 63 papers were featured, and there was excellent audience participation and discussion. The subjects covered the entire gamut of food science and technology, and included foodgrains. proteins, fruits and vegetables, plantation products, dairy products, animal food products, microbiology, food contaminants and additives. and sensory food quality.

Many facts and ideas were thrown up by the symposia presentations and discussions. Use of hedonic scales for sensory evaluation have tended to become trivialised: unless large numbers are used, such ratings count for little. For multidimensional quantification of sensory attributes, a useful model, of a discriminant function type, is that of Srinivasan and Shocker (1973). Once Kendall correlation data are generalised to a multivariate situation, and the data converted to scores by some technique, regular multivariate methods for parametric cases can be used. In developing a new product, it must fulfil a true need: the question then becomes one of identifying that need, and then of determining what product qualities or attributes might be expected to fulfil it. In this light, rural orientation of a product is no different from urban. Consumers in India must be activated to protect their rights from various blandishments, and there is need for a consumer protection Act, such as is prevalent in many countries, to give teeth to the other laws that already exist.

Toxicants of all kinds are an increasing source of concern. Outbreaks of several diseases in men and cattle have now been traced to the consumption of foods contaminated with fungal toxicants. This would imply far stricter monitoring of food materials by the authorities than at present, and education of the public regarding methods of decontamination, e.g. of raw groundnut oil. Many foodborne diseases are caused by microbial agents; animal food products are particularly prone to such infection, and the high degree of vegetarianism in India can be counted a blessing. While a few staphylococci more or less may not matter, salmonellae at any level are taboo; methods of quantitative assay are not too accurate and new methods are being developed. An increasing degree of environmental pollution has meant more metallic contaminants in food, water and air, with consequent outbreaks of even serious illness in advanced countries. India is no exception; lead, arsenic, cadmium and mercury levels in foodstuffs are causing concern, and maximum limits have been laid down in the PFA rules. Particularly serious are such practices such as the deliberate coating of turmeric pieces with yellow lead chromate to heighten the colour and conceal insect damage, and the high levels of lead in vegetables grown along busy roadways and subject to motor car exhausts. With pesticides, contamination may occur both by direct application or indirectly from adjacent crops or atmospheric fallout. Persistence of pesticides is particularly a problem with chlorinated hydrocarbons which are popular because they have a broad spectrum of activities. The answer may lie in using natural compounds to manipulate the insect population without leaving toxic residues.

Processing to some degree is essential before foodgrains can be consumed; any resulting nutritional loss is important because foodgrains are consumed in

volume. Where wholemeal *atta* is used the problem is less severe, but with a cereal like rice, it is desirable to promulgate a deliberate policy to promote undermilling, and implement it on a national scale. Recent work has thrown new light in such areas as unavailable carbohydrates (which in the soyabean are as high as 30 to 40 percent), essential fatty acids, and on binding of many nutrients and antinutrients which render them unavailable. New methods to improve nutrition have become available, such as the fortification of suitable vehicles, partial foodstuff processing to lengthen shelflife and aid distribution, better packaging to improve nutrient retention, and the like. Both fermentation and drying represent low-cost methods of greatly improving nutritional quality and digestibility, converting virtually inedible materials into acceptable human foods. Recent interest in solar drying has given a new impetus to traditional drying techniques, and useful future technologies may emerge. In considering irradiation as a food preservation method, it is worth remembering that products generated by radiolysis are no different from those found in foods subject to accepted processing techniques such as heat sterilisation. The recent clearance for many irradiated foods by an international committee must be viewed in conjunction with reports of positive dominant lethal mutagenicity in animals fed freshly irradiated material, first noticed at the National Institute of Nutrition, Hyderabad and now confirmed by laboratories in West Germany and Denmark.

Table 1—Topics for Symposia.

SYMPOSIUM 1: SENSORY EVALUATION AND CONSUMER PERCEPTIONS

1.1 V. S. Govindarajan, *Problems and possibilities of sensory evaluation.*
1.2 G. Sadasivan, *Some new methods of sensory evaluation.*
1.3 Rajni Chadha, *Consumer concepts and product development.*
1.4 J. Mandanna, *Consumer perceptions of food quality.*

SYMPOSIUM 2: MICROBIAL, METALLIC AND MAN-MADE FOOD CONTAMINANTS

2.1 S. G. Srikantia, *Mycotoxins in foods.*
2.2 J. W. Bhattacharjee, *Microbial contaminants of foods.*
2.3 O. P. Kapur and K. V. Nagaraja, *Metallic food contaminants.*
2.4 P. G. Deo, *Pesticide contamination and biodegradability.*

SYMPOSIUM 3: NUTRITIONAL ANGLES TO FOOD TECHNOLOGY

3.1 H. S. R. Desikachar, *Some aspects of the effects of processing on the nutritive value of foods.*
3.2 D. V. Rege, *Some changing concepts of nutrition in relation to food technology.*
3.3 Sunit Mukherjee, *Low-cost food processing possibilities.*
3.4 P. K. Vijayaraghavan, *Experiences with irradiated foods.*

Bombay Chapter

A two-day seminar on 'Convenience Foods: Opportunities and Challenges' was held on December 20-21, 1980 at the Centaur Hotel, Bombay. About 250 delegates participated in the seminar.

The seminar was inaugurated by Dr. K. K. G. Menon, Director, Hindusthan Lever Research Centre and the inaugural address of Dr. A. Sreenivasan which was read out in his absence surveyed the possibilities and prospects of the convenience food industry in India and emphasized the aspects of quality and self-constraint on pressure advertising.

The first session chaired by Dr. C. L. Nagarsekar included three papers. Dr. S. D. Dharkar spoke on the recent advances in Thermally Processed Convenience Foods. The topic of Bakery and Confectionery Products was discussed jointly by Shri M. G. Sathe and Mr. J. C. Shah. Dr. H. S. R. Desikachar delineated the work done at the CFTRI, Mysore, on grain-based convenience foods for Indian kitchen. Mr. M. A. Tejani explained his experiences with the pre-mixes manufacture and marketing.

Session II chaired by Dr. M. V. Rajgopal included four papers. Dr. S. Govindarajan spoke on the Role of Frozen Foods in Hotel Industry and on developing pre-cooked frozen foods. Dr. P. K. Vijayaraghavan gave an account of the convenience foods developed for defence personnel. Dr. R. Jayaram, read the paper on Extrusion Technology: Applications in Convenience Foods by Dr. Ing. Marco Manzini of Pavan Spa, Italy. Dr. R. K. Baisya presented technical aspects of instant food mixes.

Third Session on Packaging and Machinery was chaired by Mr. L. K. Shah. Mr. M. R. Subramanian discussed the practical aspects of packaging of convenience foods. Dr. N. Balasubramanian presented his experiences with packaging of pre-mixes. Mr. R. Wadwani discussed the advantages of a horizontal filling and packing machine for convenience foods.

Session IV was about Quality and Marketing of Convenience Foods. It was chaired by Dr. K. A. Savgaon. Dr. Mrs. Sumati R. Mudambi in her paper on Nutritional Aspects of Convenience Foods, deplored the lack of interest in nutritional considerations and emphasized the need for laying down nutritional standards for food products. Dr. S. Ranganna presented the methodology employed for quality control of convenience foods. Shri N. M. Parikh, discussed Food Laws and Convenience Foods. The problems of marketing convenience foods were discussed by Mr. N. P. Singh.

Dr. S. P. Manjrekar chaired session V on Food Additives which had three papers: Role of Food Additives in Convenience Foods by Dr. U. Y. Rege, Additives in Relation to Texturising Convenience Foods by Prof. D. V. Tamhane, and Deep-fat Fried Foods by Dr. R. Jayaram.

Dr. P. R. Krishnaswamy emphasized the need for planning the development of convenience foods taking into consideration the fuel famine in villages. Convenience foods needing less energy at the users' end should therefore, be encouraged. Prof. D. V. Rege commended the organizations like Lijjat Papad and Apna Bazaar who have introduced new convenience foods for mass consumption. He also commended the small-scale manufacturers who ventured to market new products based on innovative concepts.

Delhi Chapter

The new office bearers of the Chapter are:

President	:	Shri P. N. Narang
Vice-President	:	Dr. V. B. Mitbunder
Hon-Secretary	:	Dr. S. K. Roy
Hon-Treasurer	:	Dr. D. S. Khurdiya
Councillor	:	Dr. N. K. Dadlani

The Chapter has organised two special lectures, the first by Dr. Susanta K. Roy on "Post Harvest Technology of Fruits and Vegetables in United Kingdom" on 26th September 1980 and another by Dr. K. T. Achaya on "Vegetable Oil Production System in India on 3rd November 1980.

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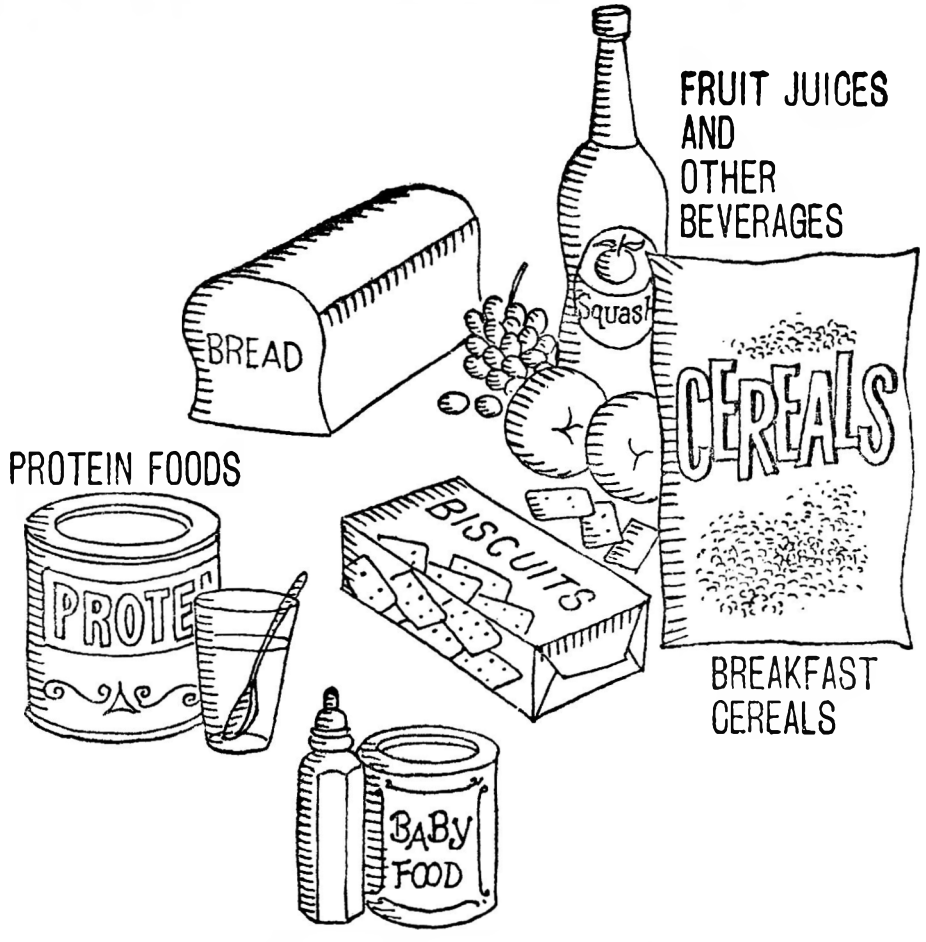

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1. **REVIEWS IN FOOD SCIENCE AND TECHNOLOGY — VOL. IV**
   Royal 8vo, hard bound, P. 255.
   Price: India **Rs. 8/–**
   Abroad: **$5 Surface mail**
   $8 Air mail.

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

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

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   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.

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

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   (*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:* As in (*c*), if the article is not published then the title of the article should be mentioned.

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

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

9. Consult the latest copy of the *Journal* for guidance.

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Research Papers

Research Notes

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