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Physical Alteration in Eggplant Fruits During Storage at Different Temperatures

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A study was carried out on alterations produced in eggplant fruits during storage at different temperatures. Physical parameters of fruits were determined on the initial day and compared with the results obtained after 18 days of storage at 5°, 10° and 20°C. The results obtained indicated that preservation of these fruits during 18 days at 10°C produced less variation in their physical characteristics compared with the other conditions studied (5° and 20°C). The preservation at 5°C showed symptoms of chilling injury. Therefore, we consider that the optimum conditions for preservation are from 5° to 10°C during less than 18 days.

The eggplant (*Solanum melongena* L.) is one of the most important vegetables grown in India as brinjal. It is available throughout the year and is used primarily as a cooked vegetable. Its tropical origin makes its sensitive to low temperatures, appearing in its fruits the typical symptoms of chilling injury. Therefore, one of the problems of these vegetables is their conservation. The symptoms of chilling injury in fruits include discoloration and the pitting of peels. These diseases have been reported in apples[1], bananas[2] and other fruits[3,4].

There is little information on the chemical constituents of eggplant fruits[5,6]. In view of its importance in Indian diet and its potentialities as a raw material in the making and dried food industries, it was considered desirable to ascertain the nutritive value of these fruits, which can provide information that might be of use to breeders, consumers and food technologists.

The data on dry matter, loss of weight, colour, firmness, elasticity and anthocyanins during storage are reported here.

Materials and Methods

Eggplant fruits (*Solanum melongena* L. cv. 'Black Round'), obtained from a farmer in the South East of Spain (Almeria) were used for this study. Fruits of the same size, weight and shape were harvested. Groups about 2 kg (10 fruits) were placed in trays covered with perforated plastic sheet. Seven trays were placed at each temperature. Temperature and relative humidity of the chambers were 5°C with 75 per cent; 10°C with 80 per cent; 20°C with 55 per cent (ambient conditions). The determinations of the constituents were made on the initial day and at 4, 7, 11, 14, 18 and 21 days after harvest (corresponding to 0, 3, 6, 10, 13, 17 and 20 days of storage).

Weight loss is expressed in percentage from difference between the weights of fruits before and after the storage.

Firmness and elasticity measures are the mean of six determinations and were carried out with an Instron Food Testing Instrument mod. 1140, with penetration until structure breaking, 10 kg of weight, chart time drive JY-JX 800 mm/min, punch of approximately 4 mm of diameter. Firmness is expressed in kg/cm^2. Elasticity is expressed as the slope obtained when the weight applied is represented against the time needed to break the structure.

Colour of peel fresh fruits was determined by HunterLab Colour Difference Meter D-25-2. Colour readings were expressed by three parameters. *L*, a measure of lightness on a scale from "0" (black) to "100" (white); *a* which denotes greenness when negative and redness when positive; *b*, denoting blueness when negative and yellowness when positive. *L*, *a* and *b* are derivatives of the most fundamental C.I.E. values, namely X, Y and Z. The measure was carried out on discs of the same size as the cell of the Hunter Lab to avoid the light dispersion.

Anthocyanin content of the fresh peel was determined by the method of Nothmann *et al.*[7], and expressed as absorbance values at 550 nm of methanolic extract of peel of fresh fruits. The other method used for determination of anthocyanin content was a colorimetric determination based on the decolouration of these compounds at pH 3.5. The absorbance was expressed as mg of cianidine per 100 ml of methanolic extract.

Water content was determined by drying the samples (previous trituration) at 60°C for 24 hr and then raising the temperature up to 105°C to a constant weight.

Statistical analysis: All determinations were carried out on three replications. LSD for temperatures and samplings were calculated using an analysis of variance with a significance of 1 per cent and 5 per cent. The results obtained for each temperature were adjusted with a mathematical formula to the most probable curve.

Results and Discussion

The results obtained during the storage of eggplant fruits at different temperatures indicated the important variations that take place in the physical characteristics depending on the temperature and the period of storage.

After 7 days of storage at 20°C, a decrease in the commercial quality with respect to physical appearance is observed, and this quality was lost after 11 days storage. It is precisely in this last phase, when chilling injury appeared in fruits stored at 5°C. Similar findings were reported by Nothmann[8]. At 10°C, the water loss was more with more microbial growth with respect to the other two studied temperatures. This finding is supported by Abdel-Maksoud *et al*[9]. These micro-organisms limited the period of storage, that was of 10-15 days in the fruits conserved between 5-10°C.

The observed weight loss (Fig 1) at 5° and 10°C produced a quality loss due to the wilting of the fruits after 17 days of storage, at 20°C this was more pronunced and it was accompanied with a clear decrease of the water content of the fruits due to the temperature and to the relative humidity of the chamber. Munoz-Delgado[10] reported that a weight loss from 3 to 6 per cent might cause dehydration, wilting or wrinkling in certain fruits, leading to a pronounced reduction of their commercial quality.

The ascending tendency of the evolution curves of firmness (Fig 2) at the three temperatures up to ten days of storage can be explained by the water loss at constant speed during this period of time. This fact can be observed in Fig. 1,

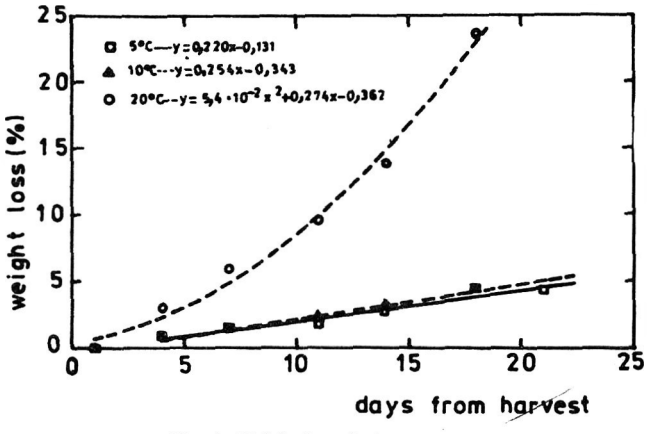

Fig. 1. Weight loss during storage.

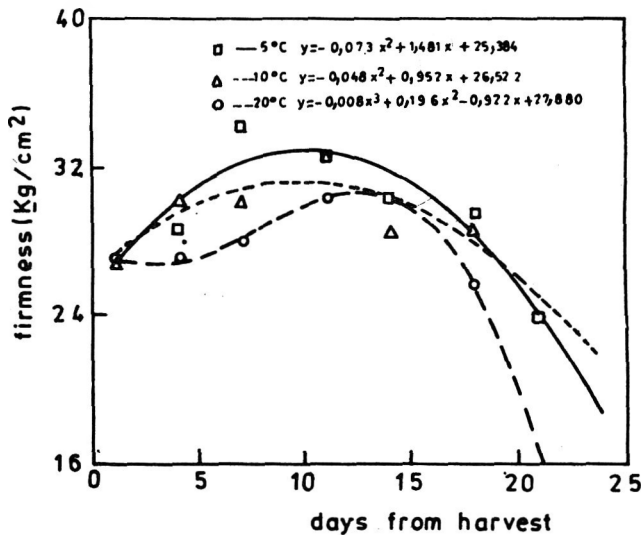

Fig. 2 Changes in the firmness during storage.
$LSD_{5\%}(T^a)=0.20$ $LSD_{1\%}(T^a)=0.27$
$LSD_{5\%}(S)=0.30$ $LSD_{1\%}(S)=0.40$

corresponding to weight loss. After this period of time, a decrease in firmness can be observed. According to Aubert and Pochard[11] this decrease is caused by degradation reactions produced during the storage, consisting, mainly, in the transformation of pectin substances into more soluble forms. It is clear that the temperature influences the elasticity of the fruits, which increases as the temperature and period of storage increase.

The colour measurement is one of the main tests used to assess the product quality. Parameters *L*, *a* and *b* are shown in Table 1.

The suitability of the parameters *L* and *b* could be observed to establish the appearance of pitting after 13 days of storage at 5°C.

The *b* parameter is very interesting because it affects the blue tonalities that appear in the fruit due to the presence of anthocyanin pigments. The changes of its values from negative to positive observed in the fruits stored at three temperatures and at harvest indicate the degradation produced in the anthocyanins during this period (Table 2). The fruits conserved at 10° and 20°C did not present variations during the experimentation period. However, the fruits stored at 5°C for 13 days indicate an evolution to the yellow tonalities due to the appearing of the pitting.

The decrease in the anthocyanin content observed up to seven days of storage, due to the chemical lability of these compounds, is followed by an increase during a period of 7 days, probably caused by synthesis from glucids because of the activation of the metabolism of eggplant fruits stored at 10° and 20°C. This was not seen in fruits stored at 5°C, because of a slow metabolism due to the low temperature.

TABLE 1. CHANGES IN HUNTER PARAMETERS (L, a, b) DURING STORAGE

Hunter colour values	Storage temp (°C)	Days from harvest 1	4	7	11	14	18	21
L	5	6.09	6.71	6.49	7.94	7.43	9.89	11.05
L	10	6.09	8.32	5.94	6.39	7.92	5.99	
L	20	6.09	7.16	5.91	6.64	8.56	6.47	
		$LSD_{5\%}(T)$= 0.26 $LSD_{5\%}(S)$=0.36		$LSD_{1\%}(T)$=0.35 $LSD_{1\%}(S)$=0.49				
a	5	3.55	3.69	5.52	5.86	4.57	3.14	4.47
a	10	3.55	6.18	3.80	5.28	7.32	2.84	
a	20	3.55	4.77	4.10	5.84	6.08	4.20	
		$LSD_{5\%}(T)$=0.34 $LSD_{5\%}(S)$=0.48		$LSD_{1\%}(T)$=0.46 $LSD_{1\%}(S)$=0.65				
b	5	—0.71	—0.42	0.53	—0.63	—0.34	1.82	5.08
b	10	—0.71	1.64	—0.98	0.06	1.17	0.36	
b	20	—0.71	0.11	0.21	0.26	0.54		

TABLE 2. CHANGES IN THE ANTHOCYANINS CONTENT DURING STORAGE

Storage temp. (°C)	D.O. at 550 nm after indicated days from harvest 1	4	7	11	14	18	21	Cianidine (mg/100 ml) after indicated days after harvest 1	4	7	11	14	18
5	1.81	1.49	0.44	0.78	0.58	0.23	0.79	1.90	1.54	0.32	0.58	0.70	0.32
10	1.81	1.21	0.91	2.05	1.87	1.96		1.90	1.23	0.90	2.29	2.46	2.13
20	1.81	1.04	0.31	1.82	1.95	1.10		1.90	1.15	0.37	1.97	2.49	0.89
		$LSD_{5\%}(T)$=0.06 $LSD_{5\%}(S)$=0.08			$LSD_{1\%}(T)$=0.09 $LSD_{1\%}(S)$=0.11			$LSD_{5\%}(T)$=0.08 $LSD_{5\%}(S)$=0.12			$LSD_{1\%}(T)$=0.11 $LSD_{1\%}(S)$=0.16		

References

1. Lyons J M and Breidenbach R W, Chilling injury, in *Post-harvest Physiology of Vegetables*, by Weichmann J. M Dekker Inc, New York, 1987, 305.
2. Marriot J, Bananas-Physiology and biochemistry of storage on ripening for optimum quality, *CRC Crit Rev Food Sci Nutr*, 1980, 13, 41.
3. Jones R L, Freebairn H T and McDonell J F, The prevention of chilling injury, weight loss reduction and ripening retardation in banana, *J Am Soc Hort Sci*, 1978, 103, 219.
4. Morris L L, Chilling injury of horticultural crops: An overview, *Hort Sci*, 1982, 17, 161.
5. Jaiswal S P, Kaur G, Bindra V and Singh J, Physical characteristics and chemical constituents of the fruits of important varieties of brinjal, *Indian Fd Packer*, 1974, 28, 10.
6. Bajaj K L, Kaur G, Chadha M L and Singh B P, Polyphenol oxidase and other chemical constituents in fruits of eggplant (*Solanum melongena* L.) varieties, 1981, 8, 37.
7. Nothmann J, Rylski I and Spigelman M, Color and variations in color intensity of fruit of eggplant cultivars, *Sci Hort*, 1976, 4, 191.
8. Nothmann J, Eggplant, in *Fruit Set and DEvelopment*, by Monselise S P, CRC Press Inc, Boca Raton, Florida, 1986, 145.
9. Abdel-Maksoud M, Abou Aziz A B, Abdel-Kader A S and Abdel-Samie K A, Effect of storage temperature on quality and decay of eggplant fruits, *Egypt J Hort*, 1987 4, 119.
10. Munoz-Delgado J A, *Refrigeracion y congelacion de alimentos vegetables*, Ed. Fundacion Espanola de la Nutricion, Madrid, 1985, 5.
11. Aubert S and Pochard E, Problemes de conservation en frais de l'aubergine (*Solanum melongena* L.). II. Essais preliminaries de mise au froid, *P.H.M. Rev Hort*, 1981, 216, 35.

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Biochemical Changes During the Ripening of Jackfruit (*Artocarpus heterophyllus* L.)

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Jackfruit (Varikha group) was analysed at four ripening stages for sugars, organic acids, amino acids, lipid constituents, respiration and aroma characteristics. Fruit firmness, alcohol insoluble solids, starch, tannins, soluble amino acids, phospholipids and free fatty acids decreased during ripening. Sucrose, glucose, fructose, sugars:acid ratio, citric:malic acid ratio, total lipids, total fatty acids, unsaponifiables and total sterols increased during ripening. Sixteen amino acids were identified and their concentration varied during ripening. The same types of fatty acids were present in all stages. The respiration pattern of "bulbs" with seeds excised from mature fruits followed a typical climacteric rise while ripening. Initiation of aroma production coincided with the rise in respiration and maximum aroma production accounted during the post-climacteric decline in respiration. The volatiles collected at ripe stage were resolved by GLC to 38 components.

Jackfruit, a tropical fruit tree native to India is known to produce the large size fruit weighing as much as 50 kg. It bears fruits on the deciduous branches arising on the trunk and on the primary and secondary branches. On an average in ripe jackfruit, the bulbs, seeds and rind form 29, 12 and 59 per cent of the bulk respectively[1]. The ripe fruits are eaten as a desert fruit and unripe fruits cooked and eaten as vegetable. The fleshy pericarp of individual bulbs on ripening develops a characteristic aroma. Studies on the biochemical changes in jackfruit during ripening are not reported in the literature. The present paper is, therefore, concerned with changes in sugars, organic acids, amino acids, lipid constituents, respiration and aroma characteristics during post-harvest ripening.

Materials and Methods

Fruit sample: The jackfruit analysed belonged to the firm and fleshy pericarp (Varikha) group. Jackfruit collected from trees grown at the experimental orchard, Hessaraghatta were used for this study. Fruits were harvested when spikes on the skin well separated and skin colour turned to brownish yellow. Harvested fruits were kept at ambient temperatures (25 $\pm$ 2°C) and at 70 $\pm$ 5 per cent relative humidity for ripening. The edible bulbs were separated from the rind. The fleshy pericarp of bulb minus seed was used for analysis. The fruits were analysed at four ripening stages viz., at harvest maturity (0 day), ½ ripe (2 days after harvest), ¾ ripe (4 days after harvest) and eating ripe stage (8 days after harvest). Each sample consisted of 2 fruits of same maturity stage. There were two independent samples for each maturity stage.

Methods: Firmness of whole fruit was measured using fruit pressure tester model P.T. 327. For estimating pulp colour (carotenoid pigments), weighed quantity of pulp was extracted thoroughly with ethanol: petroleum ether 60-80° (1:1 ratio) mixture. The combined solvent extract after removing water layer was dried over anhydrous sodium sulphate, made upto a known volume and absorbance was measured at 448 nm. Fruit pulp was extracted with 80 per cent ethanol in a Soxhlet extractor. The alcohol insoluble residue was used for starch estimation[2]. The extract after evaporating alcohol was used to estimate sugars, organic acids and amino acids. The methods employed for fractionation and estimation were the same as reported[3] previously. A combination of cationic and anionic exchange column chromatography was used to fractionate and purify the constituents. For measuring total acidity, a known weight of fruit pulp was blended with distilled water and made upto a definite volume. An aliquot was titrated against standard alkali and acidity expressed as citric acid. Tannins in water extract were estimated using Folin — Dennis reagent[2]. Ascorbic acid content was estimated by 2, 6 dichlorophenol indophenol dye titration method[2]. Thiamine and riboflavin were estimated by AOAC methods[2]. Nitrogen content was estimated by micro-kjeldhal's method and the values multiplied by 6.25 was taken as protein content. A known amount of oven-dried sample was digested with tri-acid mixture and the digested material was used to estimate minerals[4,5]. The phosphorus was estimated by Vanadomolybdate yellow colour method, iron by orthophenanthroline colorimetric method and potassium and calcium using Flame photometer.

Contribution No.276/88 of Indian Institute of Horticultural Research, Bangalore — 560 089.

Total lipids were estimated according to the procedure described by William *et al* [6]., phosphorus content in an aliquot of total lipid was determined[7] and approximate percentage of phospholipid was obtained multiplying the phosphorus content by twenty five[8]. Free fatty acid value, total fatty acids, unsaponifiables and squalene were determined following AOAC methods[2]. For total sterols estimation, the unsaponifiable fraction adsorbed in alumina column after elution with hexane for squalene estimation was thoroughly washed with ethanol:ether (3:1 ratio) mixture. The ethanol : ether extract was evaporated and the residue dissolved in chloroform. The content of sterols in chloroform extract was estimated by Lieberman Burchard[9] reaction. Methyl esters of fatty acids were prepared by reacting the fatty acids with Boron trifluoride-methanol reagent[10]. Methyl ester in heptane was analysed by GLC (Toshniwal gas chromatograph) equipped with flame ionisation detector and a stainless steel column (6' × ⅛" O.D.) packed with 10 per cent DEGS + 1 per cent H_3PO_4 on chromosorb W (AW) 80 to 100 mesh (Altech associates Inc. Illinois U.S.A.) and nitrogen at a flow rate of 60ml/min was used as the carrier gas. Chromatographic separation was achieved at column temperature of 180°C. Injector port and detector were maintained at 260°C and 250°C respectively. The identity of each fatty acid peak was established by their relative position on the chart with that of known mixture of reference standards (Sigma U.S.A.). Gas chromatographic peak areas were determined by multiplying peak height by peak width at half height. The per cent composition of individual fatty acids were calculated as per AOAC procedure[2].

Aroma characteristics of fruit was evaluated by sensory evaluation. The intensity of aroma was recorded in a five point scale as (−) no aroma, (+) mild aroma, (++) moderately strong aroma, (+++) strong aroma and (++++) very strong aroma. For volatiles fractionation by gas chromatography, the macerated pulp in water (1:1 ratio) was subjected to conventional steam distillation in Clevenger's apparatus. The distillate obtained was extracted with peroxide free diethyl ether. The solvent extract was dried over anhydrous sodium sulphate and the oil was recovered after removal of solvent in a flash evaporator at room temperature. The essential oil was analysed by GLC equipped with a flame ionisation detector and a stainless steel column (6' × ⅛" O.D.) packed with 10 per cent SE 30 on chromosorb W—HP 80 to 100 mesh and nitrogen at a flow rate of 60 ml/min was used as the carrier gas. Chromatographic separation was achieved at column temperature of 60°C for 5 min, then temperature programmed at 6°C per min to a maximum of 180°C and isothermal for 5 to 10 min at 180°C. Injector port and detector were maintained at 260°C and 250°C respectively. The quantity of the separated compounds was determined from peak area.

Respiration measurements of "bulb" including seed were made using Pettenkoffer[11] method. Each sample consisted of 10 bulbs. There were four independent samples. Respiration measurements were started immediately after separation of the bulbs from fruit (harvest maturity) and continued at one day interval till the bulbs reached eating ripe stage (8th day after harvest). The values in Fig.1 represent the average of four independent measurements.

Results and Discussion

Data shown in Table 1 indicate that fruit firmness decreased considerably as a result of ripening. From harvest maturity to ½ ripe stage, little softening was observed. The intensity of fruit softening increased from ½ ripe to ¾ ripe stage and in ripe fruits. A two fold increase in pulp colour (carotenoid pigments) was observed from harvest maturity to eating ripe stage. Dry matter, alcohol insoluble solids and starch contents decreased with ripening. Sucrose formed the major sugar followed by fructose and glucose. A three fold increase in sucrose concentrations was observed during ripening. Similarly, the concentration of glucose and fructose increased six and five fold respectively. Compared to other fruits, the total titrable acidity in jackfruit was low and it showed little changes during ripening. Sugar : acid ratio during ripening increased because of increased total sugars. Jackfruit is reported to be essentially a carbohydrate material and useful as a source of energy when consumed[1]. The low acidity level and high free sugars are responsible for the sweet taste of jackfruit. Citric and malic acids were the major non-volatile organic acids identified. Their concentration increased till ¾ ripe stage and declined in ripe fruits. The decline was much more for malic acid resulting in increased citric:malic acid ratio in ripe fruits.

Total soluble amino acid concentration was fairly high in jackfruit as compared to other edible fruits and their concentration decreased during ripening. The paper chromatographic separation of soluble amino acids indicated no qualitative changes in their pattern. Sixteen amino acids tentatively identified as α-aminobutyric acid, aspartic acid, asparagine, cystein, glutamic acid, glutamine, glycine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine and tyrosine were fractionated in all chromatograms. The concentration of individual amino acids changed from mature stage to eating ripe stage (Table 2). Asparagine. aspartic acid, glutamine, glutamic acid, glycine, α-aminobutyric acid, phenylalanine and proline formed the major soluble amino acids. Jackfruit also contained very little tannins and their concentration decreased during ripening. The edible portion at eating ripe stage had (in per cent) 5.8 mg vitamin C, 39 μg thiamine, 126 μg riboflavin, 238 mg, nitrogen (1.49% crude protein), 68 mg phosphorus, 19 mg calcium, 88 mg potassium and 0.7 mg iron.

Total lipids, total fatty acids, unsaponifiables and total sterols increased whereas phospholipids and free fatty acids decreased during ripening. Squalene content increased from harvest maturity to ½ ripe stage and then decreased till eating

TABLE 1. CHANGES IN BIOCHEMICAL CONSTITUENTS IN RIPENING JACKFRUIT

Constituents	Values at indicated days after harvest			
	0	2	4	8
Fruit firmness (kg/cm^2 pressure)	12.0	11.5	7.5	6.5
Pulp colour (O.D. at 448 nm)	0.103	0.143	0.181	0.203
Dry matter (%)	30.1	26.3	24.8	23.9
Alcohol insoluble solids (%)	8.1	4.1	3.9	3.4
Starch (%)	2.03	1.54	1.01	0.69
Sucrose (%)	2.9	7.2	7.3	8.7
Glucose (%)	0.8	4.3	4.8	4.9
Fructose (%)	1.3	5.9	5.7	6.6
Glucose : fructose ratio	0.64	0.73	0.84	0.75
Total acidity (as citric acid %)	0.21	0.22	0.21	0.13
Sugar : acid ratio	23.3	78.8	85.5	153.2
Citric acid (mg %)	51.0	87.9	92.1	67.2
Malic acid (mg %)	68.5	85.2	83.5	39.7
Citric : malic acid ratio	0.74	1.03	1.10	1.69
Total soluble amino acids* (mg%)	396	386	333	210
Tannins (mg %)	6.4	3.1	2.8	2.3
Total lipids (%)	0.247	0.276	0.320	0.339
Unsaponifiables (%)	0.049	0.047	0.055	0.055
Phospholipids (mg %)	42.3	40.1	37.8	32.9
Free fatty acids as acid value	26.3	29.8	24.6	23.2
Total fatty acids (%)	0.139	0.135	0.175	0.169
Total sterols (mg %)	5.0	8.0	8.4	9.7
Squalene (mg %)	0.34	0.86	0.68	0.51
Fruit aroma	—	+	+++	++++
Volatiles (%)	—	Trace	Trace	0.02

*as iso-leucine
— No aroma; + Mild aroma; ++ Moderately strong aroma; +++ Strong aroma; ++++ Very strong aroma
Values are the average of two independent determinations

TABLE 2. CHANGES IN SOLUBLE AMINO ACIDS IN RIPENING JACKFRUIT*

Amino acid (mg %)	Values at indicated days after harvest			
	0	2	4	8
Cystein	0.12	0.08	0.09	0.08
Aspartic acid	9.26	6.40	15.18	10.91
Lysine	0.34	0.35	0.37	0.35
Glutamic acid	3.98	2.10	6.57	2.53
Serine	0.14	0.08	0.11	0.16
Asparagine	4.13	2.85	1.52	1.15
Glycine	2.30	0.97	2.04	0.53
Methionine	0.69	0.14	0.18	0.34
Glutamine	6.41	4.92	3.81	5.33
Tyrosine	0.69	0.14	0.18	0.34
α-aminobutyric acid	5.48	1.70	1.85	2.90
Phenylalanine	17.18	14.27	16.84	12.43
Leucine	1.61	1.84	1.74	1.75
Isoleucine	1.83	1.25	0.73	0.47
Threonine	0.72	0.35	0.90	0.53
Proline	2.69	4.82	2.87	2.98

*Values are the average of 2 independent determinations

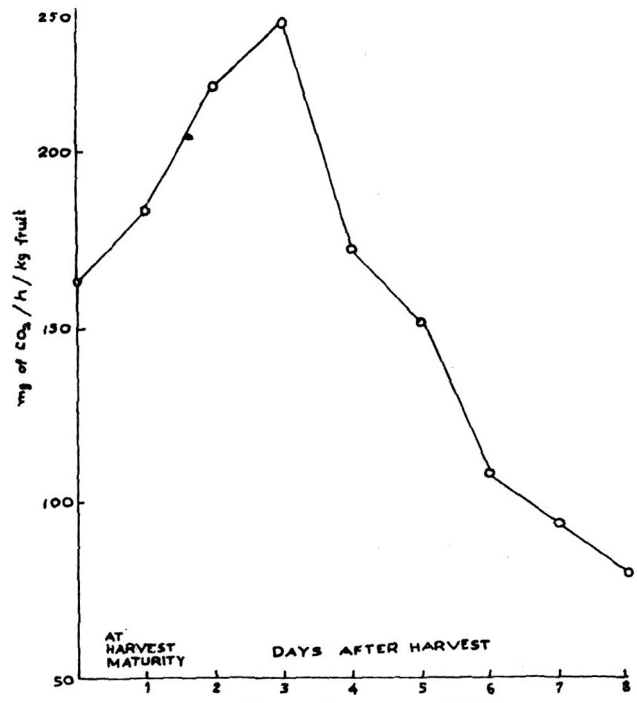

Fig. 1 Respiration pattern in ripening jackfruit

TABLE 3. CHANGES IN FATTY ACID COMPOSITION IN RIPENING JACKFRUIT*

Days after harvest	Fatty acid composition (per cent)							
	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
0	0.40	3.93	10.25	10.85	5.46	7.79	5.83	5.20
2	0.37	7.75	6.28	7.74	5.27	6.40	4.59	3.68
4	1.36	7.84	6.99	7.04	4.73	5.22	4.31	2.68
8	2.34	7.74	9.38	4.18	4.31	4.07	2.58	1.57

*Values are the average of two independent determinations

ripe stage. The decreased free fatty acids content in ¾ and eating ripe fruits indicated their role as a potential source for synthesis of other metabolites including aroma compounds during ripening. The fatty acid composition of jackfruit lipids at four ripening stages is given in Table 3. The chromatographic profile was similar in the four ripening stages studied. The fatty acids tentatively identified from the chromatogram were lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acids. The concentration of individual fatty acids changed during ripening. Unsaturated fatty acids namely palmitoleic, oleic, linoleic and linolenic acids decreased during ripening. Maximum decrease was observed for palmitoleic, linoleic and linolenic acids. Except palmitic, the other saturated fatty acids namely lauric, myristic and stearic acids did not show much changes during ripening.

The respiration pattern of "bulbs" with seeds excised from mature fruit exhibited a typical climacteric rise on ripening (Fig. 1). Climacteric peak in respiration was reached three days after harvest, followed by a steady decline until 8th day when fruit reached the eating ripe stage. Biale and Baccus[12], while studying the respiratory pattern of tropical fruits of Arizona Basin grouped jackfruit respiration of an "intermediate type". Sensory evaluation of aroma of ripening jackfruit revealed that at harvest maturity, the fruit had no aroma (−) (Table 1). The complex characteristic aroma was noticed at ½ ripe stage (+) which increased considerably (+++) at ¾ ripe stage, reaching maximum concentration (++++) at ripe stage. Initiation of aroma production coincided with respiration from pre-climacteric minimum reached to climacteric peak (3rd day after harvest). Maximum production of aroma was, however, accounted during the post-climacteric decline (4th to 8th day after harvest) in respiration. The volatiles (0.02 per cent) extracted from ripe jackfruit pulp was resolved into 38 components by gas chromatographic studies.

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References

1. Bhatia B S, Siddappa G S and Lal G, Composition and nutritive value of jackfruit, *Indian J Agric Sci*, 1955, **25**, 303.
2. *Official Methods of Analysis*, Association of Official Agricultural Chemists, Washington D C, 1965, 10th Edn.
3. Selvaraj Y, Suresh E R, Divakar N G, Randhawa G S and Negi S S, Sugars, organic acids, amino acids and invertase activity of juice from 22 grape varieties, *J Fd Sci Technol*, 1975, **12**, 75.
4. Jackson M L, *Soil Chemical Analysis*, Prentice Hall of India Pvt Ltd., New Delhi 1967.
5. Piper C S, *Soil and Plant Analysis*, Hans publishers, Bombay 1966.
6. William M K, Hanson A P and Purcell A E, Lipid of cured centennial sweet potato, *J Fd Sci*, 1971, **36**, 795.
7. Harris W D AND Propet P. Determination of phosphorus content in lipids. *J Am Oil Chem Soc*, 1954, 31, 124.
8. Kaimal T N B and Lakshminarayana G, Lipid changes in maturing *Ceiba pentandra* seed, *Phytochem*, 1972, **11**, 1617.
9. Sperry W H and Webb M, A revision of Schoenmeimer-spery method for chloesterol determination. *J Biol Chem*, 1950, **187**, 97.
10. *Official and Tentative Methods*, American Oil Chemists Society, Illinois, 1973, 3rd Edn.
11. Vogel A I, *A Textbook of Quantitative Inorganic Analysis*, Longmans Green & Co. Ltd., Great Britain, 1961.
12. Biale J B and Baccus D E, Respiratory patterns in tropical fruits of Arizona Basin, *Trop Sci*, 1970, **12**, 93.

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Changes in Sugars, Organic Acids, Amino Acids, Lipid Constituents and Aroma Characteristics of Ripening Mango (*Mangifera indica* L) Fruit

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Fruits of 7 mango cultivars viz., 'Alphonso', 'Banganapalli', 'Dasheri', 'Fazli', 'Langra', 'Suvarnarekha' and 'Totapuri' harvested at mature green stage were analysed at four ripening stages for sugars, organic acids, amino acids, lipid constituents and aroma characteristics. The biochemical changes taking place at the ripening phase included reduction in the content of starch, increase in soluble sugars and reduction in titrable acidity. Sucrose, glucose and fructose were the main soluble sugars identified and their concentration increased during ripening. Glucose:Fructose ratio showed marked differences during ripening depending on the cultivar. Malic acid concentration changed marginally and 2 to 6 fold decrease in citric acid concentration was observed during ripening. Paper chromatographic separation of soluble amino acids indicated quantitative maturation pattern changes amongst the cultivars. Eighteen amino acids were identified. Total lipids, unsaponifiables, total sterols and total fatty acids increased whereas free fatty acids and phospholipids decreased during ripening. A relationship between intensity of ripe fruit aroma and pulp colour (carotenoid pigments); sugar:acid and citric:malic acid ratios was established which could be useful in preliminary screening of mango cultivars for their eating quality. The volatiles extracted from ripe mango fruits were resolved into 50 components in 'Alphonso'; 43 in 'Banganapalli'; 46 in Dasheri', 32 in 'Fazli', 26 in 'Langra', 37 in 'Suvarnarekha' and 30 in 'Totapuri' by gas chromatographic studies.

Mango, one of the most popular and delicious fruits of major importance has several hundred distinct cultivars. Unlike other fruits, each mango cultivar on ripening has distinguishing characteristics and flavour differences. The inherent aroma of mature green mangoes on ripening changes to a strong characteristic fruity aroma in some cultivars and to a mild or blend aroma in others. The majority of the documented research in mango is concerned with cultural and horticultural aspects. Studies on the composition of ripe mango cultivars[1,2] and a few on compositional changes during growth and development are reported in the literature[3-7]. The present paper is concerned with changes in sugars, organic acids, amino acids, lipid constituents and aroma characteristics during post-harvest ripening of seven commercially important mango cultivars.

Materials and Methods

Mango fruits of 7 cvs. were collected from trees grown at the experimental orchard, Hessaraghatta. Fruits collected at harvest maturity were kept at ambient temperature (25±2°C) and at 70±5 per cent relative humidity for ripening. Each cv. was analysed at four ripening stages viz., at harvest maturity (0 day), ½ ripe (pulp colour turning stage, 2 days after harvest), ¾ ripe (pulp colour fully yellow but not ripe, 4 days after harvest) and eating ripe stage (6 to 8 days after harvest depending on the cv.). Each sample consisted of 10 fruits of same maturity stage. There were two independent samples for each maturity stage.

Edible pulp after the removal of peel and stone was composited and used for analysis. Fruit pulp was extracted with 80 per cent ethanol in a soxhlet extractor. The alcohol insoluble residue was used for starch estimation. The extract after evaporating alcohol was used to estimate sugars, organic acids and amino acids. A combination of cationic and anionic exchange column chromatography was used to fractionate and purify these constituents. The method employed for fractionation and estimation of sugars, organic acids and amino acids were the same as reported earlier.[8] For measuring total acidity, a known weight of fruit pulp was blended with distilled water and made upto a definite volume. An aliquot was titrated against standard alkali and acidity expressed as citric acid.

Total lipid in fruit pulp was estimated according to the procedure described by William *et al*[9]. Phosphorus content in an aliquot of total lipid was determined[10], and approximate percentage of phospholipids were obtained multiplying the phosphorus content by twenty five[11]. Free fatty acids as acid value, total fatty acids, unsaponifiables

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and squalene were determined following standard AOAC[12] methods. For total sterols estimation, the unsaponifiable fraction adsorbed in alumina column after elution with Hexane for squalene estimation was thoroughly washed with ethanol : ether (3:1) mixture. The ethanol : ether extract was evaporated and the residue dissolved in chloroform. The content of sterols in chloroform extract was estimated by Lieberman-Burchard[13] reaction.

Aroma characteristics of whole fruits were evaluated by sensory evaluation. The intensity of aroma was recorded in a five point scale as no aroma (−), mild aroma (+), moderately strong aroma (++), strong aroma (+++) and very strong aroma (++++). For volatiles fractionisation by Gas liquid chromatography, ripe fruits were washed thoroughly and pulp separated from peel and seed. Pulp (1 kg) was squeezed with hand and mixed with distilled water (1:1 ratio). The macerated pulp was subjected to conventional steam distillation in Clevenger's apparatus. The distillate obtained was extracted with peroxide free diethyl ether. The solvent extract was dried over anhydrous sodium sulphate and the oil was recovered after removal of solvent in a flash evaporator at room temperature. The essential oil was analysed by GLC (Toshniwal Gas Chromatograph) equipped with a flame ionization detector and a stainless steel column (10'×⅛" O.D.) packed with 10 per cent carbowax 20 M on Diatomite W (AW) 100 to 120 mesh (Altech Associates Inc, Illinois, U.S.A.) and nitrogen at a flow rate of 60 ml/min was used as the carrier gas. Chromatographic separation was achieved at column temperature of 60°C for 5 min followed by 6°C per min to 180°C and then isothermal for 5 to 10 min. Injector port and detector were maintained at 260°C and 250°C respectively. The quantity of the separated components were determined from peak area.

Results and Discussion

The main ripening trends observed in mango cultivars are presented in Tables 1 and 2. The biochemical changes taking place at the ripening phase in mango fruit included

TABLE 1. CHANGES IN AROMA, STARCH AND SUGARS OF RIPENING MANGO FRUIT

Cultivar	Days after harvest	Fruit aroma	Starch (%)	Sucrose (%)	Glucose (%)	Fructose (%)	Glucose to fructose ratio
Alphonso	0	−	4.25	0.11	6.32	1.83	3.45
	2	+	2.08	4.46	7.02	2.17	3.24
	4	++	1.33	4.50	7.07	2.62	2.70
	6	++++	0.43	4.91	7.23	3.25	2.22
Banganapalli	0	−	4.08	0.10	2.32	2.21	1.05
	2	+	2.85	0.47	3.26	2.69	1.21
	4	+	1.86	1.57	4.18	3.63	1.15
	7	+++	0.67	5.13	4.48	2.74	1.20
Dasheri	0	−	3.91	0.16	2.04	2.16	0.94
	2	+	1.38	0.77	6.16	3.17	1.48
	4	+	1.14	1.28	6.81	4.72	1.44
	7	+	0.67	1.55	7.78	5.62	1.38
Fazli	0	−	0.92	0.18	4.69	2.87	1.63
	2	-	0.56	0.39	5.39	4.09	1.30
	4	+	0.48	1.96	6.12	4.21	1.45
	7	+++	0.39	1.82	6.14	4.86	1.26
Langra	0	−	4.09	0.11	2.06	2.30	1.13
	2	−	1.94	1.64	5.02	4.52	1.11
	4	+	0.74	2.21	5.25	5.30	0.99
	7	+++	0.39	1.70	6.00	5.80	1.03
Suvarnarekha	0	−	3.43	0.23	1.56	0.99	1.58
	2	+	3.34	0.14	2.15	1.71	1.25
	4	++	1.78	0.17	6.90	3.51	1.96
	7	+++	0.38	3.54	7.15	3.63	1.97
Totapuri	0	−	3.62	0.09	5.40	1.67	3.23
	2	−	2.92	0.84	5.50	2.10	2.62
	4	+	0.88	1.26	5.72	2.35	2.43
	8	++	0.52	1.64	8.06	3.01	2.68

Values are the average of 2 determinations

0 = at harvest maturity; 2 = ½ ripe stage; − = No aroma; + = Mild aroma;

4 = ¾ ripe stage; 6,7,8 = at eating ripe stage; ++ = Moderately strong aroma; +++ = Strong aroma; ++++ = very strong aroma.

TABLE 2. CHANGES IN CHEMICAL COMPOSITION OF RIPENING MANGO FRUIT

Cultivar	Days after harvest	Acidity (as citric acid %)	Sugar to acid ratio	Citric acid (mg%)	Malic acid (mg%)	Citric to malic acid ratio	Total soluble amino acids (mg %)
Alphonso	0	2.78	2.97	188.1	37.5	5.02	166
	2	1.44	9.46	150.0	33.2	4.26	190
	4	0.94	15.18	122.9	49.0	2.51	183
	6	0.39	39.15	113.8	37.7	3.02	172
Banganapalli	0	2.89	1.60	124.8	31.9	3.92	135
	2	0.96	6.69	111.5	24.6	4.53	180
	4	1.13	8.84	108.5	27.5	3.94	190
	7	0.45	30.48	53.7	23.4	2.29	166
Dasheri	0	1.54	3.34	395.1	44.1	8.98	97
	2	1.23	23.31	184.0	32.3	5.75	68
	4	0.26	49.54	96.2	44.4	2.18	44
	7	0.18	81.68	80.4	48.0	1.67	54
Fazli	0	2.19	3.54	148.9	31.5	4.73	150
	2	1.17	8.37	140.8	29.9	4.70	100
	4	0.69	17.86	103.4	32.4	3.14	166
	7	0.24	52.20	95.6	45.9	2.08	161
Langra	0	1.32	3.99	500.1	78.0	6.41	136
	2	1.05	12.59	420.3	48.1	8.91	155
	4	0.47	27.12	168.0	45.2	3.73	142
	7	0.21	63.50	123.0	49.0	2.51	110
Suvarnarekha	0	3.44	0.81	188.0	20.9	8.97	135
	2	2.65	1.51	173.1	19.9	8.72	130
	4	1.69	6.29	168.3	26.0	6.48	168
	7	0.52	27.93	80.5	31.3	2.57	178
Totapuri	0	1.69	4.23	167.7	33.5	5.01	227
	2	1.43	5.90	141.0	33.0	4.28	232
	4	1.22	7.63	127.6	34.6	3.69	222
	8	0.18	71.01	103.8	49.5	2.09	241

Values are the average of two determinations.

reduction in the content of starch, increase in soluble sugars and reduction in titrable acids. The starch level reduced considerably from harvest maturity to eating ripe stage. Ripe fruits had minimum starch content. Similar decreases in starch content with increased amylase activity were reported in cv. 'Dasheri'[6] and Israeli grown mangoes[5] during ripening. The main soluble sugars identified were sucrose, glucose and fructose. All the three sugars, in general, increased during ripening. Sucrose concentration was low at harvest maturity, increased considerably during ripening phase and was in comparable concentration with that of fructose in cvs. 'Alphonso' and 'Suvarnarekha' and that of glucose in cv. 'Banganapalli', Glucose and fructose contents also increased during ripening. Similar trend in sugars during ripening had been reported for 'Keitt' mangoes[7], Israeli mangoes[5] and cvs. 'Dasheri'[6] and 'Badami'[14]. The glucose/fructose ratio showed marked differences during ripening depending on the cultivar. The ratio increased in ripe fruits of cvs. 'Suvarnarekha' and 'Totapuri', decreased in cvs. 'Alphonso', 'Fazli' and 'Dasheri' and remained more or less the same in cvs. 'Banganapalli' and 'Langra'. Previous workers have reported a constant glucose:fructose ratio for 'Kiett' mangoes[7] and decreased ratio for 'Alphonso' mangoes[3] during ripening.

Loss in acidity during ripening was indicated by decreased titrable acid contents in all seven cultivars (Table 2). A six to ninefold decrease in acidity from harvest maturity to ripe stage was observed. Ripe fruits of cv. 'Suvarnarekha' recorded maximum and cv. 'Totapuri' the minimum acidity levels. The reduction in acidity and increase in soluble sugars constituted substantial increase in sugar:acid ratio. Citric and malic acids were the major organic acids identified. A two to six fold decrease in citric acid was observed during ripening. Malic acid concentration changed marginally and the trend varied

depending on the cultivar. A large decrease in citric acid and a small reduction in malic acid concentration was reported for 'Keitt' mangoes[7] during ripening. Malic acid concentration was reported to be increased during ripening in cv. 'Badami'[14]. The large decrease in citric acid concentration observed was responsible for the reduction in citric:malic acid ratio in ripe mango fruits.

No appreciable changes were observed in the concentration of total soluble amino acids during ripening. However, there were varietal differences in the total amount present at different stages of ripening. The paper chromatographic separation of soluble amino acids indicated no qualitative changes in their pattern. In all chromatograms, 21 spots were observed. Eighteen out of 21 amino acids were tentatively identified as alanine, α-aminobutryic acid, arginine, aspartic acid, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine and valine. Presence of 12 amino acids in cvs. 'Pairi'[3] and 'Dasheri'[4], 17 in 'Alphonso'[15] had been reported. The concentration of individual amino acids changed markedly from harvest mature to eating ripe stage.

In addition to the influence exerted by sugars and organic acids on the overall flavour quality, both the content and composition of lipids may influence the fruit aroma significantly. The data on the changes in lipid components of mango cultivars during ripening are presented in Table 3. Total lipids, unsaponifiables and total fatty acids increased, whereas free fatty acids and phospholipids decreased during ripening. Alphonso had high total lipids content followed by cv. Langra. The other five cultivars had more or less the same lipid content at ripe stage. Similarly cv. 'Alphonso' also had maximum total fatty acids followed by cv. 'Langra'. 'Banganapalli', 'Dasheri', 'Fazli' and 'Suvarnarekha' had intermediate values. 'Totapuri' recorded minimum content. Ripening of 'Alphonso' mango at ambient temperature was reported to be associated with increased glyceride content and without any alteration in phospholipid content. Whereas these changes were not appreciable in 'Totapuri' mango[16].

TABLE 3. CHANGES IN MAJOR LIPID CONSTITUENTS OF RIPENING MANGO FRUIT

Cultivar	Days after harvest	Total lipids (%)	Unsaponifiables (%)	Phospholipids (mg %)	Free fatty acids as acid value	Total fatty acids (%)	Sterol (mg %)	Squalene (mg %)
Alphonso	0	0.671	0.025	58.9	162	0.358	6.4	0.34
	2	0.803	0.055	52.7	114	0.438	8.2	0.68
	4	0.785	0.060	50.1	123	0.523	8.2	0.51
	6	0.908	0.068	49.9	20	0.800	12.3	0.51
Banganapalli	0	0.305	0.036	72.7	46	0.224	4.8	0.34
	2	0.319	0.032	70.6	44	0.239	5.0	0.34
	4	0.382	0.040	62.0	42	0.291	5.9	0.34
	7	0.465	0.043	59.5	33	0.360	7.2	0.17
Dasheri	0	0.388	0.036	59.4	38	0.255	5.2	0.28
	2	0.359	0.054	56.8	50	0.269	5.9	0.23
	4	0.382	0.025	41.3	22	0.281	6.8	0.34
	7	0.451	0.036	52.3	17	0.360	7.2	0.40
Fazli	0	0.444	0.030	65.7	184	0.222	6.8	0.51
	2	0.445	0.035	46.1	81	0.245	9.2	0.17
	4	0.457	0.057	39.0	54	0.380	9.9	0.34
	7	0.388	0.053	41.3	29	0.263	9.4	0.51
Langra	0	0.425	0.049	79.0	53	0.329	4.4	0.36
	2	0.455	0.051	73.6	84	0.345	5.9	0.51
	4	0.556	0.049	87.3	32	0.466	6.7	0.34
	7	0.543	0.022	79.0	25	0.504	8.2	0.55
Suvarnarekha	0	0.263	0.025	90.5	136	0.141	4.4	0.17
	2	0.249	0.026	78.9	106	0.166	4.1	0.23
	4	0.300	0.031	72.7	80	0.199	5.4	0.28
	7	0.417	0.046	60.5	42	0.381	6.7	0.11
Totapuri	0	0.401	0.028	48.6	148	0.132	8.2	0.36
	2	0.409	0.028	48.6	148	0.132	8.2	0.36
	4	0.434	0.034	42.3	71	0.132	8.2	0.51
	8	0.445	0.033	24.4	46	0.131	8.9	0.34

Values are the average of 2 independent determination

TABLE 4. RELATIONSHIP OF AROMA CHARACTERISTICS OF RIPE MANGO FRUITS WITH PULP COLOUR, SUGAR : ACID AND CITRIC:MALIC ACID RATIOS

Cultivar	Aroma characteristics	Pulp colour (O.D. at 448 nm)	Sugar to acid ratio	Citric to malic acid ratio
Alphonso	Very strong aroma (++++)	1.30	39.15	3.02
Suvarnarekha	Strong aroma (+++)	1.20	27.93	2.67
Banganapalli	Strong aroma (+++)	1.05	30.48	2.29
Fazli	Strong aroma (+++)	1.02	52.20	2.08
Langra	Strong aroma (+++)	1.05	63.41	2.51
Totapuri	Moderately strong aroma (++)	0.85	71.01	2.09
Dasheri	Mild aroma (+)	0.59	81.68	1.67

Considerable reduction in free fatty acids was registered in all the cultivars while ripening. The role of fatty acid as an intermediate during breakdown of lipid is well known. The decreasing pattern of free fatty acids emphasises their role as a potential source for synthesis of other metabolites including aroma compounds. Earlier results on the activity of enzymes involved in the biogenesis of lipid derived volatiles in mango fruit revealed that considerable variability in activity level existed among the cultivars. The relatively high aldehyde forming activity observed in cv. Alphonso was associated with the presence of C_6 aldehydes in high concentration in this cultivar[17]. The total sterol and squalene contents in unsaponifiable fraction of lipid from four ripening stages in seven cultivars revealed that the total sterol content increased and squalene content did not show a definite trend during ripening.

Different mango cultivars at ripe stage possess a distinctive, characteristic aroma and flavour. Flavour is based mainly on the balance between sugars and organic acids and of numerous aroma compounds. Efforts were made to relate the intensity of ripe mango fruit aroma scored by sensory evaluation with that of biochemical parameters such as pulp colour (carotenoid pigments), sugar:acid ratio and citric:malic acid ratio (Table 4). Cultivar 'Dasheri' with mild aroma (+) had minimum value for total carotenoids as compared to 'Alphonso' with high total carotenoids and very strong aroma (++++). 'Suvarnarekha', 'Banganapalli' and 'Fazli' with strong aroma (+++) had intermediate values. 'Totapuri' with moderately strong aroma (++) had value lower than that of the above three cultivars but higher than cv. 'Dasheri'. Ripe fruits of cv. 'Dasheri' also recorded minimum value for citric: malic acid ratio. 'Alphonso' with maximum aroma grade (++++) had high citric:malic acid ratio. The other five cultivars had intermediate values. 'Dasheri' had maximum sugar:acid ratio. The ratio progressively decreased as the aroma grade of fruit increased. 'Totapuri' with moderately strong aroma (++) had lower ratio than 'Dasheri'. The other five cultivars had still lower values than 'Totapuri'. A correlation was reported to be ascertained between organolaptically evaluated aroma and flavour characteristics and the ratio of palmitic to palmitoleic acid in mango[16]. The relationship obtained between aroma and biochemical parameters could be useful in preliminary screening of mango cultivars for their eating quality.

The volatiles of ripe mango fruit extracted was oily in nature for cvs. 'Alphonso', 'Banganapalli', 'Langra' and 'Suvarnarekha' and waxy in nature for cvs. 'Dasheri', 'Fazli' and 'Totapuri'. The volatiles were resolved into 50 components in 'Alphonso', 43 in 'Banganapalli', 46 in 'Dasheri', 32 in 'Fazli', 26 in 'Langra', 37 in 'Suvarnarekha' and 30 in 'Totapuri'. Aroma of 'Dasheri' mango[18] was reported to be a mixture of 30 to 40 components and that of cv. 'Langra'[19] essence 21 components.

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References

1. Lodh S B, Subramanyam M D and Divakar N G, Biochemical studies on some mango varieties, *Indian J Hort*, 1974, 31, 160.
2. Kulkarni V and Rameshwar A, Biochemical and physical composition of fruits of some importtant Indian mango cultivars, *Progr Hort*, 1981, 13, 5.
3. Krishnamurthy S, Patwardhan M V and Subramanyam M, Biochemical changes during ripening of mango fruit, *Phytochem*, 1971, 10, 2577.
4. Pandey R M, Rao M M and Singh R N, Biochemical changes in the developing mango fruit (*Mangifera indica* L) cv. Dasheri, *Progr Hort*, 1974, 5, 47.
5. Fuchs Y, Peris E and Zauberman G, Changes in amylase activity, starch and sugars in mango fruit pulp. *Scientia Hort*, 1980, 13, 155.
6. Tandon D K, and Kalra S K, Changes in sugar, starch amylase activity during development of mango fruit cv. Dasheri, *J Hort Sci*, 1983, 58, 449.
7. Medlicott A P and Thompson A K, Analysis of sugars and organic acids in ripening mango fruit (*Mangifera indica* L, var. Keitt) by high performance liquid chromatography, *J Sci Fd Agric*, 1985, 36, 561.
8. Selvaraj Y, Suresh E R, Divakar N G, Randhawa G S and Negi S S, Sugars, organic acids, amino acids and invertase activity of juice from 22 grape varieties, *J Fd Sci Technol* 1975, 12, 75.
9. William M K, Hanson A P and Purcell A E, Lipid of cured centennial sweet potato, *J Fd Sci*, 1971, 36, 795.
10. Harris W D and Propat P, Determination of phosphorus content in lipids, *J Am Oil Chem Soc*, 1954, 31, 124.
11. Kaimal T N P and Lakshminarayana G, Lipid changes in maturing *Ceiba pentandra* seed, *Phytochem* 1972, 11, 161.

12. *Official Methods of Analysis*, Association of Official Agricultural Chemists, Washington D C 1965, 10th Edn.
13. Sperry W H and Webb M, A Revision of the Schoenmeimer — Spery method for cholesterol determination, *J biol Chem*, 1950, 187, 97.
14. Shashirekha M A and Patwardhan M V, Changes in amino acids, sugars and non-volatile organic acids in a ripening mango fruit (*Mangifera indica* L. Badami variety), *Lebensm — Wiss Technol*, 1976, 9, 369.
15. Subramanyam H, Krishnamurthy S and Parpia H A B, Physiology and Biochemistry of mango fruit *Adv Fd Res*, 1975, 21, 223.
16. Gholap A S and Bandyopadhyay C, Contribution of lipid to aroma of ripe mango (*Mangifera indica* L.), *J Am Oil Chem Soc*, 1975, 52, 514.
17. Selvaraj Y, Studies on enzymes involved in the biogenesis of lipid derived volatiles in ripening mango (*Mangifera indica* L.) fruit. *J Fruit Biochem*, 1989, 12, 289.
18. Kapur K L, Volatile components of Dasheri mango, *J Fd Sci Technol*, 1983, 20, 242.
19. Golap A S and Bandyopadhyay C, Comparative assessment of aromatic principles of ripe Alphonso and Langra mango, *J Fd Sci Technol*, 1975, 12, 262.

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Proteolytic and Lipolytic Degradation Products as Indicators for Quality Assessment of Canned Mutton Curry

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Quantification of quality defects such as off-odours occasionally noticed in commercially sterile canned mutton curry was studied by monitoring certain proteolytic and lipolytic degradation products such as polyamines, free amino acids and phospholipids. Canned mutton curry was prepared using fresh, spoiling and spoiled meat. Analysis of important polyamines such as putrescine, cadaverine, spermidine and histamine by TLC and HPLC and phosphorus present in phosphatidyl ethanolamine and phosphatidyl choline by TLC, densitometry and calorimetry showed that in the canned curry of acceptable quality having no off-odours, the quantities were 9.0 mg/100g curry and 500 mg/100 g curry respectively. In those samples which had off-odours and were of doubtful and unacceptable quality, the quantities of polyamines and phospholipid phosphorus were nearly 74 μg/100 g curry and less than 70 mg/100 g lipid respectively. Therefore, polyamines and phospholipid phosphorus are indicative of the quality of meat used for processing. Measurement of free amino acids (expressed in terms of tyrosine value) did not prove to be helpful.

Acceptability of canned curried meat is dependent on the bacterial quality of mutton used for processing. Canned curried meat prepared from mutton, spices, vegetable fat is a popular ready-to-eat item which is a regular supply to Indian Defence Services. Meat used in the manufacturing of processed meat products must be free from significant deteriorative flavour changes. Investigations in the last few years in our laboratory have concentrated on the study of chemical aspects of meat spoilage under warm temperature in relation to their detection in the processed meat products. Quantification of such defects particularly those which can be related to the occasional occurrence of putrid off-odours in commercially sterile canned mutton curry have been the main aim of these investigations.

It has been reported[1-4] that analysis of total free fatty acids (FFA) and certain specific fatty acids present in the FFA fraction by TLC and GLC to be useful as indicators of quality. In order to provide additional, complementary tests for quantifying such flavour defects noticeable in the end product suspected to arise from the use of bad quality meat, various heat stable chemical compounds were selected and monitored in the product prepared from meat with varying degrees of freshness. Chromatographic and photometric techniques were employed for measuring the changes in phospholipids, polyamines and tyrosine value and the data are presented in the present paper.

Materials and Methods

Chemicals: Phosphatidyl ethanolamine (PE), phosphatidyl choline (PC), phosphatidyl serine (PS), L-tyrosine, dansyl chloride (5-(dimethyl amino)-naphthalene 1-sulphonyl chloride); polyamines such as putrescine, cadaverine, spermidine, histamine were Sigma grade (Sigma Chemical Co., USA).

Equipments: Spectronic-20 (Baush and Lomb) was used for colorimetric analysis. TLC plates were scanned using microprocessor controlled Scanning Densitometer model CS-930, Schimadzu Corporation, Japan. HPLC analysis was carried out on High Performance Liquid Chromatograph (Waters Associates, USA) equipped with an automated gradient controller, U6K injector, UV 254 nm detector (440 model) and a data processor (model 730). The separation was carried out using a reverse phase (Micro Bondapak — C_{18}) column.

Preparation of samples (canned mutton curry): Canned mutton curry[2] was prepared using (a) fresh meat mince (F) (4-5 h postmortem) with a standard plate count (SPC) of $\leq 10^6$ organism/g — FMC, (b) spoiling meat (S_1) with a SPC $\sim 5\times10^7$ organisms/g — S_1MC, (c) spoiling meat (S_2) with a SPC $\sim 5\times10^8$ organisms/g — S_2MC and (d) totally spoiled meat (S_3) with a SPC $> 10^9$ organisms/g — S_3MC. Meat curry was packed in SR-lacquered 301×309 OTS cans and processed at 121°C for 1 hr. In all, two batches of curry were prepared and in each batch at least three cans of each type of sample were made and duplicate samples from each of the three cans were subjected to analysis.

Phospholipid analysis: Lipids from canned mutton curry a, b, c, d and also from raw meat mince F, S_1, S_2 and S_3 (using which the curry was prepared) were extracted[5] and quantified. 25 μl of the choroform solution of the above

(2 g lipid/10 ml) were used for separation and identification on TLC[6]. For direct quantification of individual phospholipid-phosphorus, plates were sprayed with molybdic acid and scanned in a TLC plate scanner at 580 nm. Using 0.5 mg of lipid, the total lipid phosphorus was determined by colorimetry[8].

Tyrosine value: Two g sample (a,b,c,d) was blended and extracted with 10 ml chloroform to remove the interfering lipids from the sample. The aqueous layer was deproteinised using 30 ml of 5 per cent trichloro-acetic acid (TCA) and after centrifugation the total volume was made to 40 ml with TCA solution. This solution (2.5 ml) was taken for colorimetric estimation (710 nm) using Folin-Ciocalteu reagent[9].

Analysis of polyamines: Polyamines were extracted from 90 g curry samples using 150 ml of 0.4 M perchloric acid[10]. After solvent extraction and purification, the residues were dissolved in 25 ml distilled mater. Five ml of the sample extract and 0.2 ml standard amine solution (1-10 μ moles) were converted to fluorescent dansyl derivatives[11] The crystalline residues were finally dissolved in 2 ml and 1 ml acetonitrile for samples and standards respectively. Twenty five μl of these solutions were fractionated on silica gel G plate[11] and detected under UV light (360 nm). Identities were established with the help of authentic standards. For quantification, HPLC was used. The dansyl amines (authentics) were purified on TLC prior to HPLC analysis in order to eliminate all the minor impurities that were detectable in HPLC. The solutions containing dansylated amines extracted from the meat curry were diluted to 1:10 with acetonitrile and 5 μl was applied on the C_{18} Micro bondapak column. They were separated in the isocratic mode using 0.6 per cent glacial acetic acid in water as solvent A and acetonitrile : methanol (1:1) as solvent B mixed in 15:85 proportion (1 ml/min flow rate) and detected with a UV detector 254 nm (0.05 AUFS) and quantified.

Results and Discussion

Fig. 1A and 1B give the TLC separation of the various phospholipids present in lean meat (F, S_1, S_2, S_3) undergoing spoilage and also those present in canned meat curry a, b, c and d, respectively. Four phospholipids viz. PE, PC, PS and in addition to PA (phosphatidic acid) were distinctly resolvable. The concentrations appeared to be very high in lean meat undergoing spoilage (Fig.1A) compared to that present in meat curry (Fig.1B) which is a composite mixture of lean meat, adipose, saturated vegetable fat, spices, condiments, etc. with a meat to gravy ratio of 55:45. From the iodine absorption characteristics, PE and PC appeared to be more unsaturated than PS.

Fig. 2 gives the densitometric scanning pattern of the phospholipids from curry samples separated on TLC. Fig.3 gives the changes in concentrations of different phospholipid phosphorus (PE, PC and PS) and also the total lipid

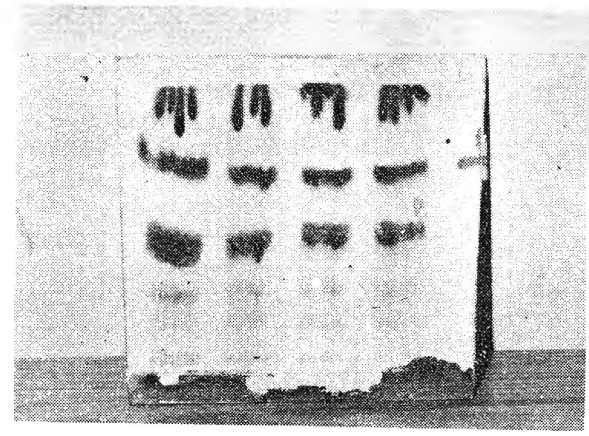

Fig. 1A: Thin layer chromatogram of phospholipids in lean meat; F — Fresh, S_1, S_2 — Spoiling, S_3 — Spoiled.

phosphorus. From the lipid phosphorus and phospholipid phosphorus contents, the quantities of the phospholipids can be directly calculated using a factor of 25. All the three phospholipids showed degradation during spoilage, PE showed nearly 45-50 per cent and PC and PS 15-20 per cent reduction in the initial stages of spoilage (sample b). Thereafter (in samples c and d) nearly 90 per cent of the remaining PE got degraded, while in case of PC and PS the degradation was about 35-40 per cent of the remaining quantities.

Igene *et al*[12] have reported decreases in PE and PC during frozen storage and cooking of beef. Decrease in PE and lipid phosphorus during spoilage or storage of meat have also been reported by various workers[13-15]. Changes in these phospholipids in canned mutton curry prepared from fresh, spoiling and spoiled meat have not been reported so far.

For polyamine analysis, TLC separation was found to be suitable for routine qualitative detection. For quantification,

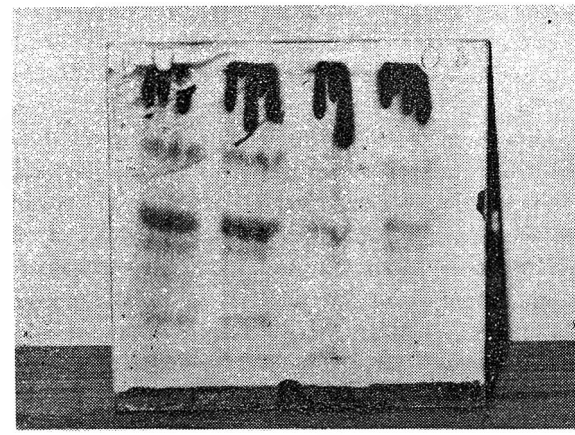

Fig.1B: Thin layer chromatogram of phospholipids in canned meat curry; a — FHC, b — S_1MC, c — S_2MC, d — S_3MC.

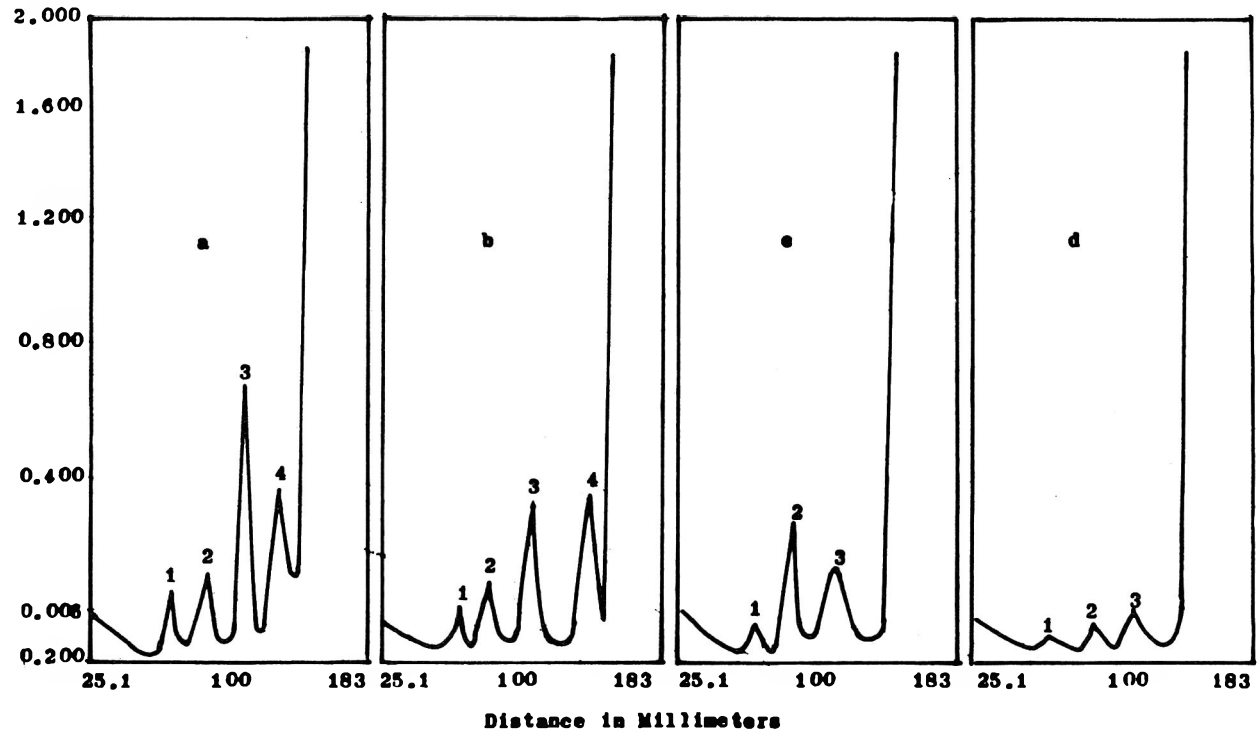

Fig.2: Densitometric scanning pattern of phospholipids separated on TLC, Spray reagent: Molybdic acid reagent; Photomode — Absorption Reflection; Wavelength — 580 nm; a, b, c, d — canned meat curries
1 — Phosphatidyl serine; 2 — Phosphatidic acid; 3 — Phosphatidyl choline; 4 — Phosphatidyl ethanolamine.

HPLC was the method of choice. Though gradient elution techniques are reported in the literature for the separation of a few polyamines[16,17], under the present conditions isocratic mode was found to give satisfactory solution. Fig.4 gives the quantitative changes in various polyamines. In the control sample (a) prepared with fresh meat, quantities of histamine and spermidine were very small (<0.5 μg/100 g curry) and cadaverine was not detectable. Putrescine was present in comparatively higher quantities (3.5 μg/100 g curry). Rogowski *et al*[18] have also reported the absence of cadaverine and presence of small quantities of putrescine and spermidine in fresh beef. When the initial SPC on meat prior to processing increased to about 10^7 organism/g as in sample b, the quantities of putrescine and cadaverine increased to about 4-5 μg/100 g but the concentrations of other amines registered a slight increase. This may be due to the limited availability of the substrate such as basic amino acids ornithine, arginine, lysine which are gradually liberated by the proteolytic activity of the microbial enzymes. As the spoilage progressed as in sample c, there was a tremendous increase in the putrescine and cadaverine concentrations (nine to ten folds) indicating the more availability of the substrate due to the increased rate of proteolysis by which time the off-odour begins to be evident in the product and cannot be totally disguised by spicing and processing. It is the product of S_2MC (sample c) that normally poses problems as sometimes it is difficult to rely exclusively on the presence of slight off-

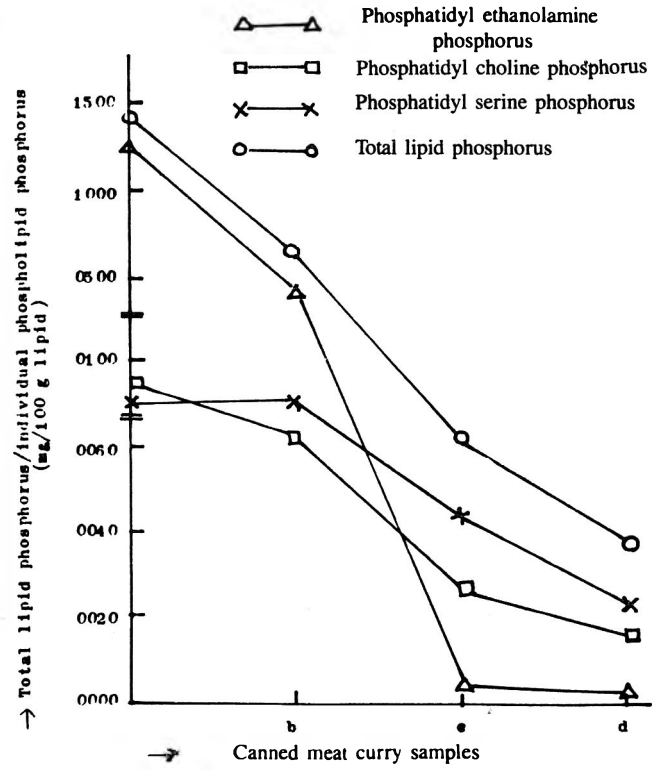

Fig. 3: Changes in total lipid phosphorus and different phospholipid phosphorus as determined by colorimetry and TLC—scanning densitometry respectively.

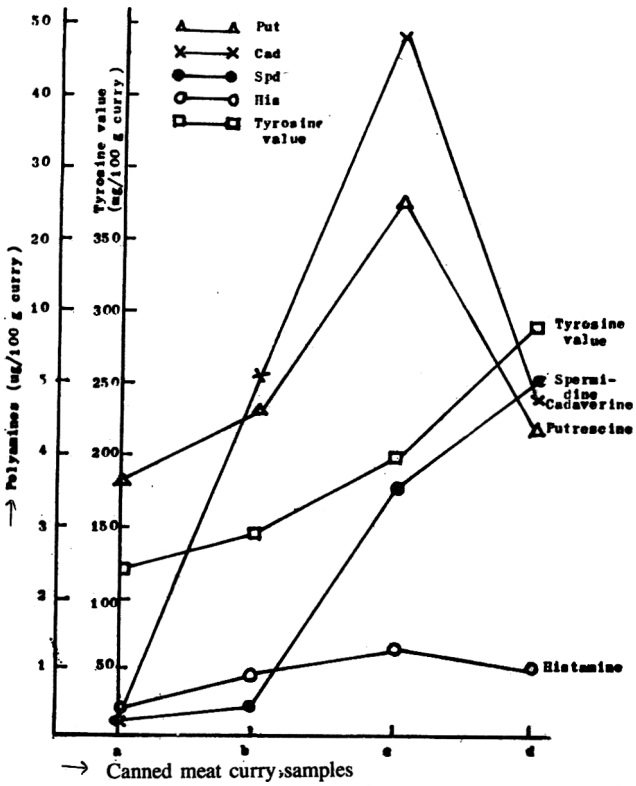

Fig.4: Changes in tyrosine value and some important polyamines present in canned meat curry samples

odours which leads to doubts with regard to acceptance or rejection. In sample d, which gave foul smell, though the quantities of various amines remained higher than that found in samples a and b, it was found to be lesser compared to sample c. This could not be explained since this was not traceable as sampling error. This might be due to other metabolic conversions. From our experience, if a meat which has an initial SPC range of 10^8—10^9 is used for processing, the off-odour cannot be masked and the quality can be established by various chemical markers. Wortberg *et al.*[19] have also reported that at incipient stages of spoilage of minced beef and pork, the value of cadaverine rose sharply from 0.5 to 9.6 mg per 100 g. From these observations it is evident that polyamine concentration can also be used as a marker for the detection of use of spoiling meat for meat processing.

Fig.4 also depicts the alterations with respect to tyrosine value (another indicator of proteolytic activity). Measurement of tyrosine value revealed that there was a progressive increase in the tyrosine value. Between samples a to b and from b to c slight increase (20-28 per cent) was noticed, thereafter about 40-42 per cent increases in samples c and d were noticed, indicating the increased rate of proteolysis in samples prepared with meat containing more than 10^8 organisms/g. Though tyrosine value has been reported to be a suitable quality testing indicator for fish[20], this was not found to be very reliable for canned meat curry samples due to narrow range (112-288 mg/100 g curry) over which the changes occurred, causing overlapping of samples.

The above results indicate that both lipolytic and proteolytic products are useful as indicators of quality of meat used for processing.

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References

1. Vasundhara T S, Kumudavally K V and Sharma T R, Altered free fatty acid levels in fresh or canned mutton as indicators of spoilage, *J Fd Prot*, 1983, 46, 1050.
2. Kumudavally K V, Vasundhara T S, Joyce D'Souza and Sharma T R, Changes in certain free fatty acids as an aid for the detection of spoiled mutton used for processing meat curry, *J Fd Technol*, 1984, 19, 653.
3. Vasundhara T S, Kumudavally K V and Sharma T R, *Quality of Mutton Used in Canned Products : A Simple Rapid Chromatographic Procedure for Assessment. A Preliminary Working Manual*, Defence Food Research Laboratory, Mysore, India, 1986.
4. Vasundhara T S, Kumudavally K V, Joyce D'Souza, Phanindra Kumar H S and Sharma T R, Chemical indicators for determining the pre-sterilization quality of canned curried meat : Factors affecting the quantity of the chemical indices, *Fd Chem*, 1988, 29, 133.
5. Folch J, Lees M and Stanley G H S, A simple method for the isolation and purification of total lipids from animal tissues, *J Biol Chem*, 1957, 226, 497.
6. Skipski V P, Peterson R F and Barclay M, Thin layer chromatography of phospholipids, *J Lipid Res*, 1962, 3, 467.
7. Stahl E, *Thin layer chromatography — A Laboratory Handbook, Reagent No.166*, Springer — Verlag, New York Inc., 1969.
8. Fiske N and Subba Row Y, The colorimetric determination of phosphorus, *J Biol Chem*, 1925, 66, 375.
9. Pearson D, Application of chemical methods for the assessment of beef quality, II Methods related to protein breakdown, *J Sci Fd Agric*, 1968, 19, 366.
10. Spinelli A M, Laknitz L and Wasserman A E, Effect of processing on the amine content of pork bellies, *J Agric Fd Chem*, 1974, 22, 1026.
11. Sherma J and Touchstone J C, *Densitometry in thin layer chromatography — Practice and application*, John Wiley and Sons, New York, 1979, 241.
12. Igene J O, Pearson A M and Gray J I, Effects of length of frozen storage, cooking and holding temperatures upon component phospholipids and the fatty acid composition of meat triglycerides and phospholipids, *Fd Chem*, 1981, 7, 289.
13. Fishwick M J, Changes in the lipids of turkey muscle during storage at chilling and freezing temperatures, *J Sci Fd Agric*, 1968, 19, 440.
14. Goklap H Y, Ockerman H W, Plimpton R F and Peng A C, Qualitative alterations of phospholipids in beef patties cooked after vacuum and nonpackaged frozen storage, *J Fd Sci*, 1981, 46, 19.
15. Sharma N, Kowale B N and Joshi H B, Effect of cooking and storage on phospholipids in broiler, *J Fd Sci Technol*, 1982, 19, 1.
16. Hui J Y and Taylor S L, HPLC determination of putrefactive amines in foods, *Ass. off. analyt. Chem. J.* 1983, 66, 853.
17. Mietz J L and Karmas E, Chemical quality index of canned tuna as determined by HPLC, *J Fd Sci*, 1977, 42, 155.
18. Rogowski B and Dohle I, Determination of biogenic amines in meat and meat products, *Mitt. Bund. Fleish.*, Kulmbach, 1983, 81, 5601.
19. Wortberg B and Woller R, Quality and freshness of meat and meat products as related to their content of biogenic amines, *Fleishwirtschaft*, 1982, 62, 1457.
20. Bradley H C and Bailey B E, Estimation of decomposition of fish muscle, *Fd Res*, 1940, 5, 487.

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Bacterial Flora of Some Fishes of Maharashtra and Saurashtra Coasts (India)

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Native microflora in different varieties of fishes available along Maharashtra and Saurashtra coasts in India were studied during 1983-87. The flora of fishes studied were *Saurida tumbil, Nemipterus, Lepturocanthus savala, Chirocentrus dorab* and *Coilia dussumeri*. The groups of bacteria, belonged to *Vibrio, Acinetobacter, Pseudomonas, Micrococcus, Sarcina, Flavobacterium* and *Cytophaga* species. Sometimes anaerobes were also isolated from the fish intestines.

Generally, the flesh and the internal organs of healthy and freshly caught fish are bacteriologically sterile. In contrast, the external slime/skin and the digestive tract of feeding fish contain qualitatively and quantitatively variable bacterial flora, which play a major role in the spoilage of fishes. Different investigators have shown that species belonging to the genera *Vibrio, Pseudomonas, Acinetobacter, Moraxella, Flavobacterium, Cytophaga, Micrococcus, Corynebacterium* as major flora and *Proteus, Sarcina* and *Bacillus* as minor flora[1-12] are usually present. The tropical fishes are reported to harbour mainly Gram positive bacteria[6,11]. Karthiayani and Iyer[10] reported *Achromobacter, Vibrio, Pseudomonas* and *Flavobacterium* as major flora on the slime and in the intestines of oil sardines . The major flora of oil sardines and mackerals consisted mainly of Gram negative rods belonging to the asporogenous genera *Vibrio, Pseudomonas, Moraxella, Acinetobacter, Flavobacterium* and *Cytophaga* spp.[12]. The available information on the bacterial colonisation relates to studies conducted in geographically different regions.

The current study was intended to provide information on the profile of bacteria associated with intestine, slime/skin and gills of some species of fishes of Maharashtra and Saurashtra coasts during 1983-1987.

Materials and Methods

Fresh fish of varieties Mandeli *(Coilia dussmieri), Karli (Chirocentrus dorab)*, Ribbon fish (*Lepturocanthus savala*), Chor bombil (*Saurida tumbil*) and Rani (*Nemipterus* spp.) were brought to the laboratory within two hours from various boats landing at Bombay (Maharashtra) and Veraval (Saurashtra) region during 1983-87. The bacterial colonies were isolated using Sea Water Agar (SWA) at 30°C after 48 hr incubation. A representative of each colony appearing on the plates containing 30 to 70 colonies was selected. The isolates (1171) were purified and maintained by the periodic transfer on the Sea Water Agar and stored at refrigerator temperature (6-7°C) following 24 hr. incubation at room temperature (30+ 2°C). Standard methods were used to characterise aerobic bacteria[6,13]. The isolates were identified[14]. Anaerobic sulphite reducers (Clostridia) were enumerated on tryptone sulphite cycloserine (TSC) medium[15]. These were surface plated and incubated at 46°C for 24 hr and the sulphite was reduced by *Clostridium* spp. to sulphide, which precipitaed as iron sulphide (Fe_2S_3). These black colonies were presumptive of *Clostridium* and further confirmed by additional tests.

Results and Discussion

Bacterial generic profile in this study represents approximate estimate of actual population. Because the single growth medium, Sea Water Agar could not be expected to support the growth of all types of bacterial species. It has been appraised that magnitude of bacteria in one gram of sample contents oscillates between the range of 1×10^3 and 4×10^8. Similarly, it was also reported that the variation in the nature of microflora in the fish are caused by various environmental factors[16]. This dependence and variation is particularly distinct for the pelagic fishes in relation to quantitative changes of plankton in the environment[4].

Tables 1 to 5 represent the generic distribution of bacteria on the skin/slime, gills and intestine of *Coilia dussumieri, Chirocentrus dorab, Nemipterus japonicus, Lepturocanthus savala* and *Saurida tumbil*. Since these studies were conducted throughout the year, it was found that *Vibrio, Pseudomonas, Acinetobacter, Cytophaga* and *Flavobacterium* were the predominant groups of microflora present in winter, summer and post-monsoon periods, both in Maharashtra and saurashtra regions.

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TABLE 1. PERCENTAGE DISTRIBUTION OF THE BACTERIAL GENERA ON *COILIA DUSSUMERI*

	Jan/Feb			May/Jun			Sep/Oct		
	Intestines	Slime/skin	Gills	Intestines	Slime/skin	Gills	Intestines	Slime/skin	Gills
Acinetobacter	12	15	11	11	13	18	4	14	15
Aeromonas	6	6	3	—	5	1	—	5	—
Bacillus	—	7	2	—	5	1	—	3	2
Cytophaga/ Flavobacter	—	3	4	—	2	2	—	1	5
Micrococus	15	8	7	14	5	6	15	2	9
Sarcina	4	5	3	6	2	2	6	1	5
Moraxella	5	3	9	2	2	11	6	4	9
Photobacterium	6	5	2	8	3	4	5	8	2
Pseudomonas	22	18	28	26	22	24	29	30	18
Vibrio	28	24	30	31	34	28	32	28	34
Clostridium	—	—	—	—	—	—	—	—	—
Unidentified	2	6	1	2	7	3	3	4	1

TABLE 2. PERCENTAGE DISTRIBUTION OF THE BACTERIAL GENERA ON *CHIROCENTRUS DORAB.*

	Jan/Feb			May/Jun			Sep/Oct		
	Intestines	Slime/skin	Gills	Intestines	Slime/skin	Gills	Intestines	Slime/skin	Gills
Acinetobacter	12	20	14	14	12	15	9	24	12
Aeromonas	8	2	2	3	2	1	3	1	2
Bacillus	8	2	1	12	—	2	4	3	4
Cytophaga/ Flavobacter	4	3	6	6	4	5	4	3	7
Micrococus	4	12	16	6	13	12	8	12	14
Sarcina	—	—	—	—	—	—	—	—	—
Moraxella	2	4	4	3	8	3	3	6	5
Photobacterium	—	4	1	—	2	2	2	1	1
Pseudomonas	23	20	28	22	26	20	22	24	24
Vibrio	30	32	21	27	33	32	38	26	30
Clostridium	—	—	—	—	—	—	—	—	—
Unidentified	9	1	7	7	—	8	7	—	1

TABLE 3. PERCENTAGE DISTRIBUTION OF THE BACTERIAL GENERA ON *NEMIPTERUS JAPONICUS*

	Jan/Feb			May/Jun			Sep/Oct		
	Intestines	Slime/skin	Gills	ıntestines	Slime/skin	Gills	Intestines	Slime/skin	Gills
Acinetobacter	12	19	28	10	16	21	6	19	20
Aeromonas	6	—	2	9	5	4	1	5	3
Bacillus	—	4	—	—	3	—	—	7	—
Cytophaga/ Flavobacter	3	5	—	6	9	4	5	8	5
Micrococus	4	5	2	6	8	1	7	10	2
Sarcina	8	5	3	4	4	2	2	6	1
Moraxella	4	4	2	7	3	3	2	5	3
Photobacterium	3	3	2	5	2	—	6	—	—
Pseudomonas	32	24	26	21	21	28	29	17	23
Vibrio	23	21	24	28	20	31	35	23	34
Clostridium	—	—	—	—	—	—	—	—	—
Unidentified	5	10	11	4	9	6	7	—	9

TABLE 4. PERCENTAGE DISTRIBUTION OF THE BACTERIAL GENERA ON *LEPTUROCANTHUS SAVALA*

	Jan/Feb			May/June			Sep/Oct		
	Intestines	Slime/skin	Gills	Intestines	Slime/skin	Gills	Intestines	Slime/skin	Gills
Acinetobacter	14	12	10	15	12	8	15	17	15
Aeromonas	2	3	2	1	2	—	2	1	2
Bacillus	—	2	4	—	6	9	—	4	5
Cytophaga/ Flavobacter	3	6	5	4	2	6	2	4	5
Micrococus	2	12	4	5	6	2	3	5	6
Sarcina	6	5	6	8	4	4	7	7	8
Moraxella	4	2	12	7	3	10	9	1	5
Photobacterium	3	1	—	5	3	6	4	2	2
Pseudomonas	27	25	21	38	22	19	34	17	18
Vibrio	30	30	26	29	31	15	30	24	26
Clostridium	—	—	—	—	—	—	—	—	—
Unidentified	9	1	8	3	8	2	4	8	8

TABLE 5. PERCENTAGE DISTRIBUTION OF THE BACTERIAL GENERA *SAURIDA TUMBIL*

	Jan/Feb			May/June			Sep/Oct		
	Intestines	Slime/skin	Gills	Intestines	Slime/skin	Gills	Intestines	Slime/skin	Gills
Acinetobacter	18	17	23	22	12	28	21	24	22
Aeromonas	3	3	4	1	2	2	2	4	1
Bacillus	4	—	6	6	—	6	4	—	15
Cytophaga/ Flavobacter	6	5	4	1	4	3	1	2	6
Micrococus	3	4	4	2	9	1	5	4	7
Sarcina	3	4	4	7	3	5	1	2	8
Moraxella	2	2	4	9	6	6	4	3	2
Photobacterium	6	4	4	2	3	1	4	6	8
Pseudomonas	26	20	21	25	23	25	24	19	10
Vibrio	28	34	26	18	33	33	29	26	22
Clostridium	—	—	—	—	—	—	—	—	—
Unidentified	1	5	1	7	5	0	5	5	4

During the winter period (Jan/Feb), the percentage distribution of *Vibrio* was lower than those of *Pseudomonas* (Table 3) in the intestine of *Nemipterus japonicus*, during summer and post-monsoon periods, *Vibrio* registered a higher count than *Pseudomonas* in intestine, skin/slime and gill regions. Next to *Vibrio* and *Pseudomonas* species, *Acinetobacter* showed lower values (4 per cent) during all seasons. In *Coilia dussumeri*, the *Acinetobacter* showed lower values (4 per cent) during post-monsoon period, though no reason could be ascribed to this.

In this study, it was observed that Gram positive bacteria comprised about 12-25 per cent of the total bacterial load in skin/slime during all seasons. *Bacillus* was found in the slime/skin and gills mostly on *Nemipterus japonicus*. These were absent in the intestinal flora, invariably. Similar findings were reported in several other species of fishes[8,9,17] *Micrococcus* formed major constitutent of the Gram positive bacteria in all seasons. *Pseudomonas* was found in the lowest proportion (10 per cent) on the skin/slime flora of *Saurida Tumbil* during Sept/Oct period (post-monsoon) and maximum (30 per cent) in case of *Colia dussumieri*.

Overall comparison of the bacterial generic distribution among the fishes studied showed small differences in the percentage distribution of *Vibrio* and *Pseudomonas* during three seasons. *Aeromonas*, *Sarcina*, *Moraxella* and *Photobacteria* constituted lesser proportion of the bacterial flora in *toto*. Gram negative non-spore formers constituted the major flora as reported previously for other fishes studied[12]. Different proportion in the bacterial profile is due to the combined effects of seasonal variation, generic differences and the physiological activity of fishes. *Pseudomonas*, *Acinetobacter* and *Moraxella* groups predominate as compared to *Vibrio* on temperate fishes[4,18].

Bacterial isolates from the intestines of *Lepturocanthus*

savala and *Chirocentrus dorab,* which grew on TSC medium were classified as *Clostridium*. Some organisms which appeared on TSC plates, failed to grow on subsequent subculturing. So, these were reported as false positive growth. The incidence of *Clostridium* indicates the feeding habits of fishes in particular environment as bottom feeders[19].

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References

1. Wood E J F, Studies on the marketing of fresh fish in Eastern Australia, Part III, The Bacteriology of spoilage of marine fish, *Coun Sci Res Aust Rep* 1940, 3, 1.
2. Dyer F E, Microorganism from Atlantic cod, *J Fish Res Bd Can*, 1947, 7, 128.
3. Liston J, Quantitative variation in the bacterial flora of flat fish, *J Gen Microbiol* 1956, 15, 305.
4. Liston J, The occurrence and distribution of bacterial types on flat fish, *Appl Microbial* 1957, 16, 205.
5. Georgala O L, The bacterial flora of the skin of North Sea Cod, *J Gen Microbiol*, 1958, 18, 84.
6. Shewan J M, *Fish as Food* by Bergstrom G (Ed), Academic Press Inc., New York, 1961, Vol 1, 487.
7. Liston J and Colwell R R, Host and habitat relationships of marine commercial bacteria, *'Symposium on Marine Microbiology,* Oppenheimer G (Ed.) Thomas Publishers, USA, 1963, 611.
8. Venkataraman R and Sreenivasan A, A preliminary investigation of the bacterial flora of Mackerals of West Coast, *Ind J med Res*, 1952, 40, 529.
9. Venkataraman R and Sreenivasan A, Salt tolerance of marine bacteria, *Food Res*, 1954, 19, 311.
10. Karthiayani T C and Mahadeva Iyer K, Quantitative and qualitative studies on the bacterial flora of fresh oil sardines, *Fish Technol*, 1967, 4, 89.
11. Karthiayani T C and Mahadeva Iyer K, Seasonal variations of bacterial flora of fresh oil sardines (*Sardinella longiceps*), *Fish Technol*, 1971, 8, 69.
12. Surendran P K and Gopakumar K, The Bacteriology of oil sardine (*Sardinella longiceps*) and mackeral (*Rastrelliger kanagurta)* from the tropical waters of Cochin. II. Quantitative aspects *Fish Technol*, 1983, 20, 45.
13. Skerman V B D, *A Guide to the Identification of Bacteria*,Williams and Wilkins, Baltimore, 1967, 2nd Edn.
14. Bain N and Shewan M, *The Bacteriology of Fish Spoilage and Preservation*, Progress in Industrial Microbiology by Hotchenhull, DJ (Eds.) Life Books, London, 1971.
15. Harmon S M, Kourter D A and Peeler J T, Enumeration of clostridia, *Appl Microbiol*, 1971, 22, 688.
16. Patricio Garcia-Teclo and Stanalawzaleski T, Qualitative changes in the aerobic microflora from the intestinal contents of South Baltic Cod during storage at 1-2°C, *J Fd Sci*, 1970, 35, 482.
17. Gianelli F, Poplazioni batteriche isolate nasselli dell Adriatiatice Igiene mod (Parma), 1956, 49, 613.
18. Asheohong V and Vesterhus R, Bacteriological investigation on spoilage of herring during storage, *Food Res*, 1947, 12, 55.
19. Cann D C, Wilson B B, Shewan J M and Hobbs G, Incidence of *Clostridium botulinum* type E in fish product in UK, *Nature*, 1966, 211, 5055.

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Lipid Composition of Kokum (*Garcinia indica*) and Dhupa (*Veteria indica*)

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Kokum and dhupa seed powders were treated successively with various solvent systems to obtain free, bound and firmly bound lipids. The total lipid fraction was obtained by treating the powder directly with chloroform: methanol (2:1). The lipid fractions were fractionated into, neutral, glyco and phospholipids by silicic acid column chromatography, quantitated and fatty acid composition determined. The results showed that the total lipid contents of kokum and dhupa were 49.42 and 37.82% respectively. The major portion of this lipid was free lipids comprising 85% in kokum and 56.85% in dhupa followed by bound (12.14 and 38.34%), firmly bound (2.83 and 4.76%) and very firmly bound lipids (free fatty acids, 0.04 and 0.13%). Fatty acid composition showed stearic and oleic acids as major fatty acids in all the lipid fractions.

Kokum and dhupa are tree-borne oil seeds found in many parts of India, particularly the central belts and western ghats[1-3]. The potential availability of kokum seed is about 2000 tonnes and its oil about 800 tonnes[4], dhupa seeds on the other hand is about 10,000 tonnes giving about 400 tonnes[5] of fat. Kokum fat is often used as edible fat by some tribal people in South India and has light grey or yellowish colour with a grey feel and bland oily taste . It is also used as an adulterant of ghee. Dhupa fat is very similar to Australian beef and mutton tallow. Large quantities of these two fats are presently used in the preparation of soap, candles and polishes[3]. However, these fats have greater potential for use as cocoa butter substitutes/extenders after proper modifications. These also serve as a very good source of stearic and oleic acids.

Materials and Methods

The seeds were obtained from Khadi and Village Industries Commission, Shimoga. Solvents and chemicals used were analytical grade, Sephadex-G-25 (100-200 mesh), lipid and fatty acid standards from Sigma Chemicals USA and Silica Gel G, BDH, India were used.

Extraction and purification of lipids: The seeds were finely ground in a hand mill and 25 g portions of the powdered material, in triplicate, were extracted successively with the solvents at ambient temperature to get different lipids. Extract of flour with chloroform: methanol (2:1 v/v) mixture was termed as total lipids. The extraction of very firmly bound lipids as free fatty acids was done according to Bligh and Dyer[7] after acid hydrolysis of the left-over residue. The lipids were made free from the non-lipid contaminants by passing through Sephadex G-25 column[6]. The isolation of different lipids from flour is shown in Fig 1.

Fractionation and quantitation: The purified lipid fractions were resolved on silicic acid column into neutral, glyco- and phospholipids. The individual fractions were concentrated under vacuum below 40°C using a rotary flash evaporator and quantified by gravimetric method. The values for glyco and phospholipids were further confirmed by chemical methods according to Dubois *et al.*[9] and Marinetti[10] respectively.

Preparation of the fatty acid methyl esters: Methyl esters of different lipids were prepared by trans-esterification with 5 per cent methanolic hydrochloride[11] (5 per cent acetyl chloride in specially dried methanol) for 2 hrs followed by extraction of the esters with petroleum ether (40-60°C) and dried over anhydrous sodium sulphate. The solvent was removed by flash evaporation.

Gas chromatography of the methyl esters: Fatty acid composition of the different lipids was determined using a Packard gas chromatograph; model 427, fitted with a flame ionisation detector and a stainless steel column 8' × ⅛" packed with 10 per cent diethylene glycol succinate coated on chromosorb W. Carrier gas was nitrogen 20 ml/min. Fatty acid methyl esters were separated isothermally at 180°C, keeping the temperature of the injection and the detector ports at 250°C. Identification of fatty acids was done by comparing with authentic fatty acid standards.

Results and Discussion

Total lipids on seed weight basis formed 49.42 per cent in Kokum and 37.82 per cent in Dhupa. Free lipids (FL) comprised a major portion in these lipids amounting to 42.00 and 21.50 per cent (85.00 per cent and 56.85 per cent on basis of total lipids). Bound lipids (BL) were 6.00 and 14.50 per

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cent (12 per cent and 38 per cent on total lipids) and firmly bound lipids (FBL) were 1.40 and 1.80 per cent (2.83 per cent and 4.76 per cent on total lipids) in kokum and dhupa respectively. Very firmly bound lipids (VFBL) were in trace quantities as free fatty acids (Table 1).

The fatty acid composition (Table 1) shows stearic (42.2—56.7 per cent) and oleic acids (30.9—55.6 per cent) as the predominant fatty acids in these lipid fractions. Palmitic and linoleic acids are present in lesser concentrations. In addition to this, dhupa fat fractions contain myristic acid in smaller proportions. The polar (bound) lipid fractions of both kokum and dhupa have highest concentration of stearic acid. However, the distribution of oleic acid in the different fractions varies. The polar lipid fractions of kokum and non polar fraction of dhupa have maximum of oleic acid. The unsaturated to saturated fatty acid ratio and Cal I_2 value shows that VFBL of kokum and FL of dhupa fat are more unsaturated than the remaining fractions.

The silicic acid column chromatography of different lipid fractions of kokum and dhupa obtained from free, bound and firmly bound lipids (Table 2) shows that the major portion of these lipids was neutral lipid (NL). The content of NL showed a decreasing trend with increasing polarity of the extraction solvents. Thus, FL fractions of kokum and dhupa contain as high as 92.40 and 96.00 per cent of NL compared to the bound (69.60 and 68.90 per cent) and firmly bound lipid fractions (47.20 and 29.30 per cent). Next in abundance were the glycolipids (GL) followed by the phospholipids (PL). However, the contents of these two lipid fractions increased with increasing polarity of the extraction solvent. Thus, the GL content in kokum and dhupa varied from 7.50 and 3.80 per cent in FL to 38.50 and 39.30 per cent in FBL, PL's varied from 0.01 per cent in FL's to 14.20 per cent and 31.30 per cent in FBL's.

In kokum, the stearic acid content decreased gradually in all the fractions of different lipids as the polarity of the extraction solvent increased. The stearic acid content decreased from 58.7 to 44.4 per cent in NL, 64.1 to 40.8 per cent in glyco and 38.3 to 13.5 per cent in PL's. Conversely,

TABLE 1. PERCENTAGES AND FATTY ACID COMPOSITION (% WEIGHT) OF DIFFERENT LIPIDS

Lipid		12:0	14:0	16:0	18:0	18:1	18:2	US/S ratio	Cal I_2 value
Type	%*								
				Kokum Seed					
Free (FL)	42.00 (85.00)	Tr	Tr	—	52.7	47.3	—	0.90	40.77
Bound (BL)	6.00 (12.14)	Tr	Tr	19.4	46.0	30.9	3.6	0.57	32.86
Firmly bound (FBL)	1.40 (2.83)	Tr	Tr	1.8	46.9	50.0	1.2	1.05	45.18
Very firmly bound (VFBL)	0.02 (0.04)	Tr	Tr	3.0	56.7	36.5	3.8	0.67	38.04
Total lipids (TL)	49.42	1.1	1.1	2.0	38.0	53.2	4.6	1.37	53.82
				Dhupa seed					
Free (FL)	21.50 (56.85)	Tr	1.0	6.7	36.7	55.6	—	1.25	48.90
Bound (BL)	14.50 (38.54)	Tr	1.9	9.2	42.2	37.5	9.2	0.95	48.26
Firmly bound (FBL)	1.80 (4.76)	1.0	1.2	10.7	49.1	35.4	2.6	0.61	35.01
Very firmly bound (VFBL)	0.50 (0.13)	1.2	5.6	12.9	44.2	34.9	1.2	0.56	32.76
Total lipids (TL)	37.82	1.0	1.3	6.4	40.0	42.7	8.6	1.05	41.70

*on flour weight.
Figures in parenthesis indicate weight % on total lipid basis. Trace, 0.5%
US: Unsaturated; S: Saturated
Cal I_2 Value : Calculated Iodine Value

TABLE 2. PERCENTAGES OF FRACTIONS OF DIFFERENT LIPIDS AND THEIR FATTY ACID COMPOSITION

Lipid	Fractions	12:0	14:0	16:0	18:0	18:1	18:2	US/S ratio	Cal I_2 value
				Kokum					
Free (FL)	NL (92.40%)	Tr	Tr	5.5	58.7	36.2	—	0.56	31.20
	GL (7.50%)	—	Tr	7.0	64.1	28.9	—	0.41	24.91
	PL (0.01%)	Tr	—	6.4	38.3	44.7	—	0.80	38.53
Bound (BL)	NL (69.60%)	Tr	Tr	6.5	49.4	44.0	—	0.79	37.93
	GL (17.80%)	—	Tr	8.6	44.5	46.9	Tr	0.88	40.34
	PL (11.50%)	8.2	Tr	8.5	24.5	56.6	2.3	1.43	52.77
Firmly bound (FBL)	NL (47.20%)	Tr	Tr	2.8	44.4	52.8	—	1.12	45.51
	GL (38.50%)	—	Tr	3.4	40.8	55.8	Tr	1.26	48.10
	PL (14.20%)	28.6	21.0	13.7	13.5	22.0	1.1	0.30	20.86
Total (TL)	NL (88.00%)	—	2.0	2.4	50.1	45.1	—	0.80	39.22
	GL (4.00%)	Tr	3.0	10.3	32.4	54.3	Tr	1.20	46.81
	PL (3.00%)	5.5	2.5	20.9	21.1	43.0	6.9	0.98	49.01
				Dhupa					
Free (FL)	NL (96.00%)	Tr	—	6.7	42.2	51.1	—	1.04	44.05
	GL (3.80%)	—	—	32.0	16.4	51.6	—	1.07	44.48
	PL (0.01%)	Tr	Tr	27.9	28.0	44.1	—	0.79	38.01
Bound (BL)	NL (68.90%)	—	—	10.8	42.7	46.5	—	0.87	40.08
	GL (22.60%)	Tr	4.3	26.0	17.3	52.4	—	1.10	45.17
	PL (8.00%)	1.6	3.6	28.9	15.2	32.6	18.1	1.03	59.44
Firmly bound (FBL)	NL (29.30%)	Tr	1.2	10.0	36.1	52.1	—	1.11	45.44
	GL (39.30%)	Tr	1.5	12.6	27.8	58.0	—	1.38	49.99
	PL (31.30%)	1.7	6.4	28.4	20.7	42.8	12.2	0.75	36.89
Total (TL)	NL (75.80%)	Tr	1.2	8.7	38.0	52.1	—	1.09	44.91
	GL (23.10%)	1.8	2.1	15.1	33.4	47.6	—	0.91	41.03
	PL (1.00%)	1.0	2.8	21.1	18.8	39.6	16.8	0.21	63.14

NL: Neutral lipids; PL: Phospholipids
GL: Glycolipids

Mean of triplicates
Trace, 0.5%

the oleic acid content increased in the NL (36.2 to 52.80 per cent) and GL fractions (28.9 to 55.8 per cent) only. Further, in dhupa, as the polarity of the extraction solvent increased, there were increased stearic and oleic acid contents in the GL fraction. Linolenic acid was found only in the PL fractions of bound and firmly bound lipids. The linoleic content of dhupa was several times higher than those found in kokum lipids. Among the three lipid fractions, the FBL fractions were more saturated than the other two lipid fractions as indicated by their unsaturated/saturated fatty acid ratio and calculated Iodine value. Palmitic, myristic and lauric acids were found in small quantities.

Treatment of the seed flour with different solvent systems with increased polarity showed greater penetration of the solvent into the cells. Stronger polar solvents such as chloroform:methanol (2:1) and butanol saturated with water, extracts FBL's which may be bound to carbohydrates or protein. The VFBL's were extracted by hydrolysing the residue left after treatment with butanol saturated with water with 6 N HCl in a boiling water bath. Thus, the VFBLs were entirely free fatty acids.

Lipids extracted with different solvent systems are reported to have different fatty acid composition[11-13]. A similar trend was seen in kokum and dhupa seeds. In kokum, the FL extracted with petroleum ether showed higher percentages of stearic acid than the remaining lipids. However, dhupa shows increased concentration of oleic acid in the FL fraction compared to the other fractions.

It is well established that the major portion of the fats extracted from forest seeds comprised mostly NL's[14,15]. Fractionation of different lipid classes (Table 2) of both kokum and dhupa by silicic acid column chromatography showed that NL formed the major component in all the lipids except FBL of dhupa, where the GL and PL contents were higher. It is clear from these studies that the NL content decreases and the GL and PL contents increase as the polarity of the solvent used for extraction is increased.

Studies[15-17] with the fatty acid composition of the lipids of plant sources showed that these lipids were enriched with linoleic and palmitic acids. Kokum and dhupa lipids do not contain linoleic acid; instead these lipids contain high quantities of stearic and oleic acids followed by relatively

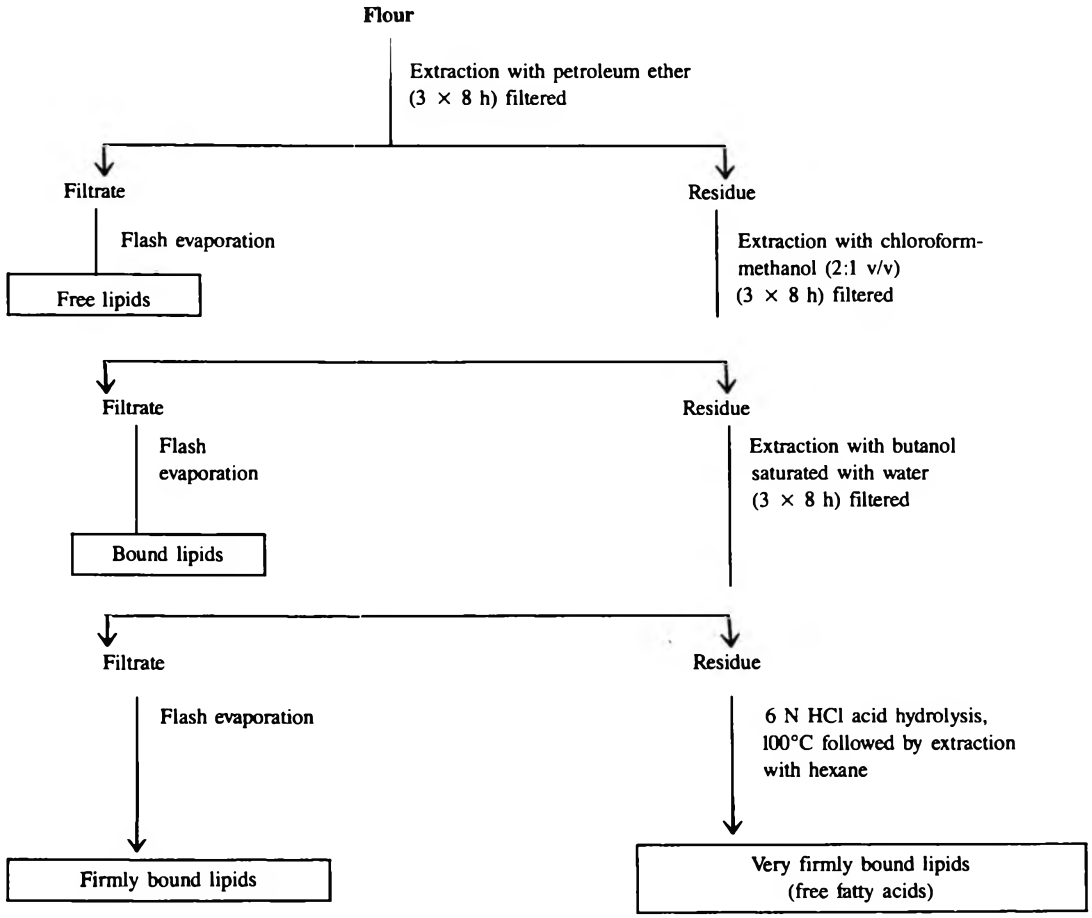

Fig. 1. Isolation of different lipids from flour.

smaller proportions of palmitic acid (Table 2). The high stearic acid content gives these fats a solid texture. They are often refined and blended with suitable unsaturated fat in preparing a substitute for cocoa butter and also for utilization as edible fat.

References

1. Report of the Sub-committee on Minor Oilseeds, Ministry of Food and Agriculture, Govt, of India, 1971, 4.
2. Report on the Special Sub-committee on Development of Minor and Non-Edible Oils, Indian Central Oil Seeds Committee, Hyderabad, 1963, 29.
3. *Plant Bearing Non-Edible Seeds* (Pamphlet), Khadi and Village Industries Commission, Vile Parle, Bombay, 1981.
4. Lakshminarayana G. Problems and prospects in processing and application of some selected vegetable butters of importance, *J. Oil. Technol Assoc India*, 1977, 9. 75.
5. Lakshmikanthan V, Solvent Extraction in India, *Extraction of Non-edible oilseeds by Solvent Method.* Dec. 1976, Regional Research Laboratory, Hyderabad.
6. Report on the marketing survey on kokum in Goa, Government of Goa, Daman and Diu, Office of the State Marketing Officer, Panaji, Marketing Series, No.7, 1981, p. 33.
7. Bligh E G and Dyer W J, A rapid method of total lipid extraction and purification *Can J Biochem Physiology*, 1959, 37, 911.
8. Wuthier R E, Purification of lipids from non lipid contaminants on Sephadex bead columns. *J Lipid Res*, 1966, 7, 558.
9. Dubois M, Gilles K A, Hamilton J K, Roberts P A and Smith F, Colorimetric method for determination of sugars and related substances *Analyt Chem*, 1956, 28, 350.
10. Marinetti G V, Chromatographic separation identification, and analysis of phosphatides *J Lipid Res*, 1962, 3, 1.
11. Kates M, Technology of lipidology isolation and analysis and identification of lipids, *Laboratory Techniques in Biochemistry and Molecular Biology* by Work T.S. and Work E. (Eds), 1972, 3, 267.

12. Shasma B N, Gopal S, Fatty acid composition of lipid classes of different varieties of Indian maize *J Res Punjab Agric Univ*, 1975, 12, 378.
13. Daga H G M, Studies in polar lipids of soyabeans *Paint India*, 1976, 26(10), 75.
14. Pin-Chiang Chen, Hsein-Yi-Sung, Fatty acid composition of the lipids in rice grains. *J Chinese Agric Chem Soc*, 1983, 21, 39. (FSTA 1985, 17, 3 M150).
15. Van Pee W M, Boni L E, Foma M N and Hendrix A, Fatty acid composition and characteristics of the kernel fat of different mango *(Mangifera indica)* varieties, *J Sci Fd Agric*, 1981 32, 485.
16. Tsuyuki H, Itoh S and Tsujimura Y, Studies on lipids in citrus seeds. I. Lipid and fatty acid composition. *Bull Coll Agric Vet Med, Nihon Univ*, 1984, No. 41, 166. (FSTA 1985(II), 4 NII).
17. Prakash Chandra K S and Chandrashekarappa G, Lipid profile and fatty acid composition of fat extracted from arils (mace) of *Myristica fragrans* and seeds of *Artocarpus hirsutus J Fd Sci Technol*, 1984, 21, 40.

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PREPARATION AND EVALUATION OF READY-TO-SERVE (RTS) BLACK CARROT BEVERAGE (KANJI)

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A ready-to-serve beverage, popularly known as 'kanji' was prepared by natural as well as controlled fermentation (*Lactobacillus plantarum*) of black carrots mixed with requisite quantities of salt, crushed mustard and red chilli powder. The changes in pH and acidity were monitored during the course of fermentation. The proximate composition of the carrots was also determined. The bottled RTS beverage was evaluated for its sensory quality and the beverage prepared by natural fermentation was rated as the best during storage for six months at room temperature.

The roots of a black variety of carrot (*Daucus carota* L.) have been used for time immemorial for the preparation of a ready-to-serve (RTS) beverage popularly known as 'kanji' in northern parts of India. The conventional method for the preparation of 'kanji' involves natural fermentation of longitudinally slit carrots suspended in a liberal quantity of water containing requisite quantity of salt, crushed mustard, and chilli powder in an earthen pot. After seven to ten days of continuous fermentation, the product acquires sufficient tartness and an attractive crimson colour. The shelf-life of the beverage is, however, limited to about seven days, and thereafter off-flavour develops. Anand[1] has worked out an improved process whereby fermentation occurs under partially anaerobic conditions. The juice is extracted by basket press and preserved by adding preservative. Although this beverage is known to possess cooling and soothing properties besides its good nutritive value[1], it has not been commercially exploited as a ready-to-serve beverage. Shah[2] attempted to prepare a 'kanji' concentrate which however, needs to be diluted prior to serving.

The present study reports the preparation of RTS beverage by lactic acid fermentation (*Lactobacillus plantarum*) of black carrots and evaluation of its sensory qualities in comparison with that of naturally fermented product.

The black carrots procured from the local market were washed thoroughly and grated in a fruit mill. A sample was withdrawn to analyse for moisture, ash, protein, sugars and fat content using standard AOAC methods[3]. The grated carrots were divided into three lots of one kg each. In lot one, one kg of grated carrots were mixed with seven kg of water, 200 g of common salt, 40 g of crushed mustard and 8 g of red chilli powder. This lot was fermented under natural conditions and served as control. The second and third lots mixed with requisite amounts of common salt, red chilli powder and water were subjected to lactic acid fermentation with or without the addition of crushed mustard respectively. These two lots were pasteurized at 73-74°C for 20 min[4], cooled and inoculated with 0.2 per cent pure culture of *Lactobacillus plantarum* containing 10^8 colony forming units/ml, activated and maintained as described previously in enriched medium for lactobacillus[5,6], the pH of which was adjusted to 7.6. All the three lots were allowed to ferment for 10 days at room temperature (20-23°C). The changes in pH and acidity were recorded during the course of fermentation.

The decanted beverage was heated to 85°C and hot filled in 380 ml glass bottles, crown corked and heat processed immediately for 25 min in boiling water, as reported by earlier workers[7,8] for carrot juice. The bottles were cooled and kept for storage studies at room temperature for a period of 9 months. Periodically, the beverage was evaluated for taste, aroma, body and overall quality by a panel of scientific workers of the laboratory, using a nine point Hedonic scale[9]. The optical density (O.D.) was measured at 515 nm after extracting the beverage (2 ml aliquot) with methanol containing 1 per cent HCl and made upto 50 ml. The black carrot slices, 2 mm thick, were dehydrated in a cross flow dehydrator initially at 70°C for 2 hr and subsequently at 60°C for 5 hr. The beverage was also prepared from the dehydrated slices during off-season as described above.

TABLE 1. PHYSICO–CHEMICAL CHARACTERISTICS OF BLACK CARROTS AND RTS BEVERAGE

	Fresh carrots	RTS beverages
Mositure (%)	88.10	—
Total ash (%)	0.48	2.90
Acid insoluble ash (%)	0.02	—
Reducing sugar (%)	4.41	1.10
Total sugars (%)	4.96	1.94
Non-reducing sugar (%)	0.52	0.80
Protein crude (%)	0.46	—
Ether extract (%)	0.20	—
O.D. at 515 nm*	ND	0.22
°Brix	ND	4.50
pH	ND	3.15
Acidity (%)	ND	0.43
Salt (as NaCl) (%)	ND	2.52

Results are on as is basis, ND = Not determined
Analysed in duplicate
*of freshly prepared and bottled beverage

TABLE 2. CHANGES IN pH AND ACIDITY OF RTS BEVERAGE DURING FERMENTATION AND THEIR SENSORY QUALITY EVALUATION

Inoculum used	Salt (%)	Mustard powder (%)	Chillies (%)	pH after indicated days						Sensory quality of the product
				0	2	4	6	8	10	
Natural	2.5	0.5	0.1	6.22	4.08	3.65	3.58	3.55	3.15	Most acceptable with colour, mouthfeel, taste and flavour etc with mustard aroma.
L. plantarum	R.Q.	R.Q.	R.Q.	6.22	4.2	3.8	3.8	3.7	3.48	Product acceptable with moderate mustard aroma.
L. plantarum	R.Q	Nil	R.Q	6.22	4.2	3.8	3.7	3.7	3.50	Not liked much due to.unfamiliar aroma.

The analytical data presented in Table 1 revealed that black carrots contain a fair amount of total sugars which are depleted by 50 per cent upon fermentation. The carrot:water ratio of 1:7 was arrived at in order to get the beverage with a tolerable acidity of about 0.3 per cent. One kilogram of grated carrots yielded 7.25 kg of RTS beverage. The left over residue was approximately one kg which contained 0.1 and 0.5 per cent reducing and total sugars respectively. The RTS beverage was found to contain 1.94 per cent sugars upon 10 days fermentation. The initial acidity (as lactic acid) of the three lots was 0.18 per cent which increased to 0.43, 0.35 and 0.30 per cent respectively, at the end of the fermentation period. The pH of the beverage dropped from 6.22 to 3.15 during the 10 days fermentation and the final values for the three lots were 3.15, 3.48 and 3.50 respectively (Table 2). The optical density of the beverage did not change much during the storage period of six months indicating no appreciable loss in colour. The optical density of freshly prepared and stored samples of RTS beverage was 0.22 and 0.20 at 515 nm respectively. This may be due to the acidic nature of the beverage. Anthocyanins, which impart deep purple colour to the beverage, are reported to be more stable in acidic medium than in alkaline or neutral medium[10].

The sensory evaluation of the three beverages revealed that the beverage prepared by natural fermentation was rated as the best followed by that prepared by lactic acid (*L. plantarum*) fermentation of the lot containing crushed mustard. Mustard imparts characteristic aroma to the finished product. Sethi and Anand[11] reported that in the fermentation of cauliflower mustard not only imparted characteristic aroma to the product but also accelerated the growth of lactic acid bacteria. The beverage prepared from *L. plantarum* inoculated lot containing no crushed mustard, offered a characteristic lactic acid flavour coupled with the aroma of volatile substances produced during fermentation and hence was not liked much by the panel. The bottled beverage as adjudged by a panel of judges using Hedonic scale[9], did not show any discernible change in colour, aroma and mouthfeel during six months storage at room temperature (13°-42°C), thereafter off-flavour began to develop. The black carrots were dehydrated to a moisture level of about 7 per cent and the dehydration ratio was found to be 5:1. The beverage prepared from dehydrated slices after 3 months storage was also found satisfactory with respect to sensory quality attributes. This suggests that 'kanji' may be made available to the consumer during off-season when fresh carrots are not available.

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References

1. Anand J C, Preparation and preservation of fermented carrot juice. *Indian Fd Pckr*, 1953, 7(2), 17.
2. Shah F H, In *Traditional Foods — Some Products and Technologies*, CFTRI Publication, Mysore, India, 1986, 14.
3. Official Methods of Analysis. The Association of Official Analytical Chemists, Washington D.C., 1975, 12th Edn.
4. Fleming H P, McFeeters R F, Thompson R L and Sanders D C, Storage stability of vegetables fermented with pH control, *J Fd Sci*, 1983, 48, 975.
5. Chen K H, McFeeters R F and Fleming H P, Complete hetrolactic fermentation of green beans by *L. Cellobiosis*, *J Fd Sci*, 1983, 48, 967.
6. Manan J K, Joshi G J, Saxena A K, Kalra C L and Berry S K, Improvement in colour of deep fat fried potato chips from cold stored potatoes by lactic acid fermentation (*L. plantarum*). *J Fd Sci Technol*, 1987, 24, 139.
7. Maur J and Maur P, What effects have the variety of carrot and processing technology applied to make semi-products upon the concentration of nutritive substances in them. *Prumysl Potravin*. 1979, 30(6), 317-18. (c.f. Kalra *et al.*, The Carrot (*Daucus carota* L.) — A most popular root vegetable — A resume Indian Fd Pckr, 1987, 41(6), 46.
8. Grewal K S and Jain S C, Physico-chemical characteristics of carrot juice beverage *Indian Fd Pckr*, 1982, 36(5), 44.
9. Amerine M A, Pangborn R M and Roessler E B, *Principles of Sensory Evaluation of Food*, Academic Press Inc. New York, 1965.
10. Markakis P. In *Anthocyanins as Food Colour*, by Markakis P (Ed) Academic Press Inc, New York, 1982, 170.
11. Sethi V and Anand J C, Effect of mustard and its components on the fermentation of cauliflower, *Indian Fd Pckr*, 1984, 38(4), 41.

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EFFECT OF CHILLING, FREEZING, THAWING AND COOKING ON PIGEON CARCASSES

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Moisture gain in one to four hours of chilling of pigeon carcasses in slush ice averaged to 6.32%. Per cent gain was not significantly different ($P<0.05$) between squabs and adults and also between males and females. Chilling time affected the moisture absorption. Average moisture loss on freezing (at —18°C) and storage (at —10°C) of carcasses for ten days was about 1.95%. The loss was lower in males than in females. Thawing and cooking losses increased with increase in chilling time.

Chilling prolongs the shelf life but results in 5-8 per cent, moisture uptake by the poultry carcasses[1]. Gain in weight during chilling and subsequent loss of moisture on frozen storage, thawing and cooking of poultry carcasses have been studied[2-11]. However, there are no such informations on pigeon carcasses in the literature. This study was, therefore, undertaken to investigate the effect of duration of chilling on freezing, thawing and cooking losses of pigeon carcasses in relation to age and sex.

One hundred and sixty squabs and adult pigeons (*Columba livia domestica*) of Indian Gola breed, respectively of 30 and 150 days of age (av. live weight 258 g) were slaughtered by improved Kosher method. Scalding temperatures and times for these were 54°C and 59°C for 60 and 90 sec respectively. Dressing and evisceration were effected as reported by Keshri and Sharma[12]. The carcasses were weighed individually before chilling (av. weight 178 g) and chilled in slush ice (ice and water in proportion of 50 per cent each. (Mickelberry *et al.*[13]) each batch contained 10 carcasses with 5 kg ice in 5 l of water) for 1, 2, 3 and 4 hr. Each bird was then reweighed (av. weight 189 g) to find out the weight gain in chilling.

Chilled carcasses were packed individually in LDPE bags (200 gauge) and allowed to freeze at —18°C. After 24 hr, the weight was recorded again. The carcasses were then packed in another bag and stored at —10°C. After ten days of storage the carcasses were removed and weight loss during frozen storage was calculated. Carcasses were then subjected to thawing in a refrigerator (at 4-5°C, RH 85 per cent) for 16 hr. Thawing loss was calculated. Thawed carcasses (av. weight 176 g) were kept in heat resistant polyethylene bags and cooked in a constant temperature water bath at 80°C (squabs 40 min, adults 60 min). Weight loss on cooking was calculated by subtracting the cooked carcass weight (av. weight 156 g) from the initial weight. Statistical analysis was done as per Snedecor and Cochran[14].

Mean values of per cent gain in weight on chilling and loss during freezing, storage, thawing and cooking of pigeon carcasses are presented in Table 1. There was no significant difference in per cent gain in weight during chilling between either squabs and adults or males and females. However, there was a significant difference in weight gain ($P<0.05$) between chilling periods. The mean values of per cent weight gain ranged from 4.07 to 7.98 in 1 to 4 hr of chilling. Kotula *et al.*[4] have reported 5 to 8 per cent of moisture uptake in broilers during chilling. Janky *et al*[7]. observed a moisture uptake of 5.09 per cent and Thomson *et al.*[15] a gain of 7.40 per cent in broiler carcasses. Marion *et al.*[16] have reported 4.72 to 5.54 per cent weight gain in turkeys. The fryers had a pick-up of 7.19 per cent. These findings are comparable to those of pigeons reported in this study.

There was a significant difference ($P<0.05$) in per cent weight loss on freezing for squabs and adults. Losses in squabs were higher than those in adults. The losses in males were significantly lower ($P<0.05$) than those in females. The chilling time had a significant ($P<0.05$) effect. The freezing loss ranged from 0.61 to 1.94 per cent. Sivacheva *et al.*[17] have reported weight changes during freezing storage of chicken meat. They found moisture losses of 2 per cent in unpacked and 0.1 per cent packed chicken during freezing. The freezing losses were possibly due to evaporation/desiccation of moisture from frozen pigeon carcasses. Frost formation was also observed within the frozen packs. Similar reasons have been assigned by Vahl[18] for the losses occurring during storage of frozen foods. There was no significant difference in loss on storage between squabs and adults. But there was a significant difference ($P<0.05$) between males and females. The loss in females was higher than that in males. There were also significant differences ($P<0.05$) between 1, 2, 3 and 4 hr of chilling. Pandey and Mahadevan[8] have reported that chilling time had a significant effect on storage losses in chickens. Keshri[6] concluded that variation in weight of carcasses, body size and surface area were some of the important factors causing differences in losses during frozen storage of poultry.

There was a significant difference ($P<0.05$) between thawing loss for squabs and adults. The loss in squabs was higher than that in adults. Also, there were significant differences ($P<0.05$) between carcasses chilled for 1, 2, 3 and 4 hr. However, there was no significant difference ($P<0.05$) between males and females. Thawing loss was between the range of 3.92 and 6.28 per cent. Although there is no report on thawing loss of pigeon meat in literature, the values obtained in this study are comparable with those reported for the broiler carcasses by Janky *et al.*[7] and Pandey and Mahadevan[8].

There was a significant difference ($P<0.05$) between cooking loss of squabs and adults. Males had a significantly

TABLE 1. PER CENT GAIN IN WEIGHT ON CHILLING AND PER CENT LOSS ON FREEZING, STORAGE, THAWING AND COOKING OF PIGEON CARCASSES

Chilling period (hr)	Squabs		Adults	
	Male	Female	Male	Female
	% gain in weight on chilling			
1	4.56 ± 0.40[a]	5.05 ± 0.41[a]	4.07 ± 0.40[a]	5.54 ± 0.36[a]
2	6.75 ± 0.21[b]	5.94 ± 0.96[b]	5.35 ± 0.26[b]	5.63 ± 0.30[a]
3	7.05 ± 0.39[c]	7.32 ± 0.45[c]	6.52 ± 0.32[c]	6.84 ± 0.41[b]
4	7.64 ± 0.54[d]	7.98 ± 0.51[d]	7.24 ± 0.25[d]	7.67 ± 0.25[c]
	% loss on freezing			
1	0.95 ± 0.17[a]	1.06 ± 0.24[a]	0.61 ± 0.13[a]	0.91 ± 0.17[a]
2	1.22 ± 0.18[b]	1.25 ± 0.20[a]	0.62 ± 0.14[a]	1.13 ± 0.05[a]
3	1.26 ± 0.13[b]	1.54 ± 0.20[b]	1.12 ± 0.19[b]	1.53 ± 0.23[b]
4	1.40 ± 0.14[b]	1.94 ± 0.13[c]	1.29 ± 0.14[b]	1.68 ± 0.30[b]
	% loss on storage			
1	0.39 ± 0.04[a]	0.59 ± 0.10[a]	0.22 ± 0.07[a]	0.55 ± 0.12[a]
2	0.41 ± 0.04[a]	0.80 ± 0.14[b]	0.61 ± 0.05[b]	0.68 ± 0.09[ab]
3	0.60 ± 0.06[b]	1.39 ± 0.25[c]	0.62 ± 0.08[b]	0.81 ± 0.16[bc]
4	0.64 ± 0.09[b]	1.53 ± 0.17[c]	0.92 ± 0.16[c]	0.99 ± 0.32[c]
	% thawing loss			
1	4.49 ± 0.44[a]	4.68 ± 0.67[a]	3.92 ± 0.39[a]	4.37 ± 0.48[a]
2	4.71 ± 0.39[a]	5.07 ± 0.69[ab]	4.22 ± 0.66[a]	4.46 ± 0.38[a]
3	6.13 ± 0.25[b]	5.48 ± 0.78[ab]	4.25 ± 0.63[a]	4.95 ± 0.76[ab]
4	6.28 ± 0.73[b]	5.64 ± 0.74[b]	5.17 ± 0.65[b]	5.65 ± 0.55[b]
	% cooking loss			
1	9.93 ± 0.74[a]	11.09 ± 0.50[a]	9.44 ± 0.66[a]	11.19 ± 1.00[a]
2	11.29 ± 1.31[b]	12.69 ± 0.77[b]	10.92 ± 0.25[b]	11.71 ± 1.20[ab]
3	12.89 ± 0.68[c]	13.10 ± 0.82[b]	11.16 ± 0.81[b]	11.89 ± 0.96[ab]
4	13.09 ± 0.52[c]	13.78 ± 0.40[b]	11.49 ± 1.29[b]	12.60 ± 0.60[b]

Each value is the average of ten replicates
± Standard error
Means in the same column with different superscript differ significantly ($P < 0.05$)

lower ($P < 0.05$) cooking loss than females. There were also significant differences ($P < 0.05$) among carcasses chilled for 1, 2, 3 and 4 hr of chilling. The cooking loss of carcasses chilled for 1 to 4 hr was in the range of 9.93 to 13.78 per cent. These values are less than those reported by Janky *et al.*[7] for broiler chickens and Pandey *et al.*[19-20] for quails.

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References

1. Kotula A W Thompson J E and Kinner J A, Water absorption by eviscerated broilers, *US Dept Agric Mktg Res Rept*, 1960, 438.
2. Froning G W Swanson M M and Benson H N, Moisture levels in frozen poultry as related to thawing losses, cooking losses and palatability, *Poult Sci* 1960, 39, 373.
3. Davis B H, Iceless pack for fresh broilers. *Broiler Business* 1963, 14, 18, 24, 26, 28.
4. Kotula AW Banwart G J and Kinner J A, Effect of post chill washing on bacterial counts of broiler chickens, *Poult Sci*, 1967, 46, 1210.
5. Gardner F and Nichols J, Product quality and consumer preference as affected by alternative methods of handling and packaging chicken, *Technical Report MRC—71-2, 1971, Texas Agril. Market Res. & Dev. Centre, Texas A. & M. University, College Station, Texas.*
6. Keshri R C, *Studies on Some Physical and Chemical Changes Occurring in Chicken Meat During Frozen Storage.* M.V. Sc. Thesis 1975, Agra University.
7. Janky D M Arafa A S Oblinger J L Koburger J A and Fletcher D L, Sensory, physical and microbiological comparison of brine-chilled, water-chilled and hot-packaged (no-chill) broilers *Poult Sci* 1978, 57, 417.
8. Pandey N K and Mahadevan T D, Effect of chilling and frozen storage on quality of chicken meat, *Indian Poult Gaz*, 1979, 63, 64.
9. Moran E T (Jr) Orr H L and Larmond E, Dressing, grading and meat

yields with broiler chicken breed, *Fd Technol*, 1970, 24, 73.

10. Swanson M H Froning G W and Richards J F, Moisture losses in frozen poultry as related to thawing losses, cooking losses and palatability, *Poult Sci.* 1962, 41, 272.
11. Klose A A Pool M F DeFremery D Campbell A A and Hanson M L, Effect of laboratory scale agitated chilling of poultry on quality, *Poult Sci.* 1960, 39, 1193.
12. Keshri R C and Sharma N, Slaughter and processing of ready-to-cook pigeons, *Poult Guide*, 1987, 24, 41.
13. Mickelberry W C Schwall D V and Stadelman W S, The effect of ice, water cool and ratios upon the moisture absorption and rate of chilling of eviscerated chicken carcasses, *Poult Sci.* 1962, 41, 1550.
15. Thomson J E Cox N A Whitehead W K Mercuri A J and Juven B J, Bacterial counts and weight changes of broiler carcasses, *Poult Sci.* 1975, 54, 1452.
16. Marion W W Jungk R A Hotchkiss D K Berg R W and Hambre M L, Class, weight and method of chilling influences on water absorption by turkeys *Fd Technol* 1968, 22, 1319.
17. Sivacheva A M Tsvetkov A I Karikh T M and Nesterov Y G, *Bull Int Inst Refri Annexe No.* 1970-3, 341.
18. Vahl L, Problems with calculation of the migration of moisture out of packaged frozen foods, *Voedingsmiddelentechnologie* 1980, 13, 20.
19. Pandey N K Mahapatra C M and Goyal, R C, Effect of various chilling methods on some quality factors of quail meat, *Indian J Poult Sci*, 1982, 17, 121.
20. Pandey N K Mahapatra C M and Goyal R C, Effect of polyphosphate in chill water on some quality factors on quail meat, *Avian Res*, 1982, 66, 56.

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MINERAL COMPOSITION OF EXPERIMENTAL FRUIT WINES

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Mineral contents of apple, plum, pear and apricot wines were determined by atomic absorption spectrophotometer. Sand pear wine had the highest contents of Na, Mg and Mn (87, 37 & 1.10 ppm resp), while max K (2602 ppm) and Zn (2.69 ppm) contents were recorded in wild apricot wine. Apple, apricot and plum wines were richest in Ca, Cu and Fe (114, 0.96, and 12.73 ppm. resp). The values determined for all the elements were in the acceptable ranges compared to those reported for grape wines.

Fruits as raw materials for wines, contribute some minerals important from processing and nutritional point of view. Among the various elements K, Ca and Mg are important for their role in human nutrition. The trace elements like Cu, Zn and Fe are analysed for their role in stabilization of wines[1] and conforming to the limits for some of the trace elements prescribed by law[2]. Similarly, analysis of Na in beverages is carried out to determine the safe limit for human consumption. Mineral composition of grape-wine is available in literature[1,3,4] but published information on this aspect is lacking for wines other than grapes. Studies to determine the mineral composition of wines prepared from various fruits were initiated and the results obtained are discussed in this communication.

Wines used for analysis were produced experimentally in the fruit technology laboratory of the Department of Post-harvest Technology.The preparation of wines from plum, pear, apple, apricot and ciders from apple were carried out according to the procedures developed for the specific fruits and the type of wine[1,5-9]. All the fruits except wild apricot were procured from the University experimental orchards. Wild apricot ('Chulli') was obtained in the form of pulp preserved with potassium metabisulphite (KMS) @ 1500 ppm. from Sharbo, district Kinnaur, where this fruit is found in abundance. The wines were prepared from cultivated apricot ('New Castle'), wild apricot ('Chulli'), apple ('Golden Delicious'), plum ('Santa Rosa', 'Green Gage' and 'Methley'), and sand pear. Cider was prepared from hpmc 7-in-1 apple juice concentrate and hard cider from 'Golden Delicious' apple. The analysis of wines for K, Na, Ca, Mg, Zn, Fe, Mn and Cu was carried out by Perkin Elmer atomic absorption spectrophotometer model 2380. Before analysis, the samples were appropriately diluted. For all determinations mixtures of acetylene gas and air were used as a fuel and oxidant respectively.

Mineral composition (Table 1) showed a wide variation in the levels of the metals in different wines. Sand pear wine had the highest contents of Mg, Na, and Mn while maximum K and Zn contents were recorded in the wild apricot wine. Wines from the other fruits were found to have intermediate mineral contents. The differences could be attributed to the inherent concentrations of the elements in different fruits as well as the procedures employed for the preparation of wines. Different methods of preparation of wines of acceptable sensory qualities from the various fruits are required because of the difference in their physico-chemical characteristics. The results also show that the Na content of the wines ranged from 11 to 87 p.p.m, the highest being recorded in pear wine and lowest in 'New castle' apricot wine. Except wild apricot, pear wine and cider, all others had Na contents less than 20 mg/l. For grape wine, the sodium content is generally less than 100 mg/l[1]. Since the levels of Na less than 300 mg/l are considered low for food and beverages, all the wines studied fall under the low Na content category[3]. The low content of sodium is a desirable attribute because high amount of it is not recommended for heart patients.

All the wines had more than 1000 p.p.m. of K the highest being in the wild apricot wine closely followed by cider and sand pear wine. Wild apricot wine, pear wine and cider had more K contents compared to the reported value of K in grape wines[1,3,4]. Therefore, these wines could be considered as good K contributing beverages. Slightly, more K in the wild apricot wine is apparently due to the KMS used for preservation of pulp from which the wine was prepared. The preservation of pulp was absolutely necessary to avoid spoilage due to distant transport. In cider, blending with fresh juice for its preparation may have accounted for high K levels and therefore, the method of blending is beneficial from mineral nutrition point of view. In plum, the differences in K contents, could be attributed to their varietal characteristics. All the wines except that of pear had Mg contents equal to or less than 25 p.p.m. while the Ca contents ranged from 71 to 139 p.p.m. The pear wine had the highest contents (37 p.p.m) of Mg. Compared to the reported values for grapes[2,3] all the wines exhibited similar Ca but low Mg content.

In wines, iron contents are normally low[1]. The Fe contents in various wines studied ranged between 2.72 and 12.73 p.p.m., Santa Rosa wine recording the highest value, and apricot ('New Castle') the lowest. These are comparable to the values mentioned in one report[1] but are considerably higher compared to those reported by other investigators for

TABLE 1. MINERAL CONTENTS (P.P.M) OF DIFFERENT FRUIT WINES

Fruit	Variety	Na	K	Mg	Ca	Fe	Cu	Zn	Mn
Apricot wine	"New Castle"	11	1481	18	71	2.72	0.96	1.92	0.88
Wild apricot wine	"Chulli"	43	2602	25	94	5.97	0.50	2.69	0.99
Apple wine	"Golden Delicious"	18	1044	16	144	3.68	0.21	0.76	0.84
Hard cider	"Golden Delicious"	19	1069	17	97	3.03	0.19	0.91	0.82
Cider	"Hpmc apple juice conc"	61	1900	23	137	4.31	0.32	1.54	1.01
Pear wine	"Sand pear"	87	1906	37	122	8.91	0.16	0.80	1.10
Plum wine	"Methley"	26	1450	20	93	4.16	0.50	1.18	0.98
Plum wine	"Green Gage"	15	1258	20	139	6.97	0.22	1.05	1.00
Plum wine	"Santa Rosa"	20	1008	18	82	12.73	0.20	1.04	0.95

grape wines[3,4]. The data show that all the wines had Cu contents less than 1 p.p.m which is a legal standard[1], the range being 0.16 to 0.96 p.p.m. The Zn levels were found to be less than 5 p.p.m, the suggested limits for grapes[1]. Cider and apricot wines contained Zn level equal to or more than 1.5 p.p.m while others recorded considerably lower values. Zinc neither has any adverse effect on wine stabilization nor proven medical effects on human beings at the levels expected in wines. But the low level of Zn is desirable as high values of it impart metallic taste to the wine. Compared to the reported values in the grape wine which range from 0.5 to 15 p.p.m, the Mn levels of the wines analysed ranged from 0.82 to 1.10 p.p.m. A reference to the 'Prevention of Food adulteration Act (PFA)', 1954[2] and the comparison of the values of trace elements like Cu and Zn of wines obtained in this study indicate that these were found to be far below than the prescribed limits.

In conclusion, it can be stated that wines produced from apple, pear, plum and apricot had the mineral contents, in the desirable limits and the fruits, are suitable for the preparation of wines with the methods employed in this study. Wines of these fruits could become good contributors of minerals.

References

1. Amerine M A Kunkee R E Ough C S Singleton V L and Webb A D, *Technology of Wine Making*, AVI. Publishing Co. Inc. Connecticut, 1980, 4th edn, 235.
2. *Prevention of Food Adulteration Act* (PFA) 1954, Eastern Book Co., Lucknow 1987.
3. Ough C S Crowell E A and Benz J, Metal contents of California wines, *J Fd Sci*, 1982, 47. 825.
4. Dayer H R and Ansher A F, Analysis of German Wines by gas chromatography and atomic absorption, *J Fd Sci*, 1977, 42, 534.
5. Vyas K K and Joshi V K, Plum wine making-standardisation of Methodology, *Indian Fd Pckr*, 1982, 36, 80.
6. Vyas K K and Joshi V K, Blending of apple wine for improving the sensory qualities. *Abst. IFCON-88, held at CFTRI*, Mysore, Feb, 18-23, 103.
7. Azad K C, Vyas K K Joshi V K Sharma R C and Srivastva M P, Utilization of sand pear-II..Effect of addition of various sugar sources on rate of fermentation and organoleptic quality of perry. *Abstract ICFOST, held at New Delhi*, June 3-5, 1986, 52.
8. Rana R S, Vyas K K and Joshi V K, Studies on the production and acceptability of cider from Himachal apples, *Indian Fd Pckr*, 1986, 40, 48.
9. *Annual Report* 1987, Department of Pomology and Fruit Technology, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) (Unpublished).

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CHEMICAL COMPOSITION OF *FLACOURTIA JANGOMAS* FRUITS

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Chemical composition of edible ripe fruits of *Flacourtia jangomas* Raeusch (*Flacourtiaceae*) grown in Tripura is reported for the first time. Chemical composition of air-dried ripe fruits (in %) was moisture, 23.05; reducing sugars, 4.2; ash, 3.46; fat, 3.2; potassium, 1.26; tartaric acid, 0.44; proteins, 0.38; phenolics, 0.30; nitrogen, 113 × 10^{-3}; iron, 18.37 × 10^{-3}; phosphorus, 16.28 × 10^{-3}; ascorbic acid, 2.12 × 10^{-1} and unidentified chemicals, 63.5 (approx). Gas chromatographic analysis of fatty acids in fats revealed the presence (in %) of palmitic, 31.3; hexadecadienoic, 3.3; stearic, 5.8; oleic, 8.0; linoleic, 4.8; α-linolenic, 44.5 and a few minor unidentified acids, 2.3. Paper chromatographic and colorimetric analysis of amino acids from aqueous alcoholic extract of the air-dried ripe fruits showed the presence (in μg/g) of proline, 8.80; hydroxyproline, 3.19; methionine, 1.17; alanine, 0.53; glycine, 0.53 and valine, 0.3. Paper chromatographic studies on simple reducing sugars and their alditol acetates indicated the presence of arabinose, glucose, fructose and galactose.

The genus, *Flacourtia* of family *Flacourtiaceae* comprises[1,2] of 15 species which are mainly found in warmer parts of Africa and Asia. Although 3 species are available in India, the only species, *Flacourtia jangomas* Rasusch (Syn. *F. cataphracta* Roxb) (local name — Paniala) is grown in Tripura. It is a small deciduous tree of 20-30 ft high with sharp decompound spines on the trunk. The leaves are pinkish or light cinnamon brown when young and ovate to lanceolate, acuminate and cremate—serrate. The fruits are ellipsoid with about 1 inch cross sectional diameter having dark red or purple colour when ripe. The fruit has a rather pleasant tart flavour. The flesh is firm, brownish green and fairly juicy. Bruising between hands renders the fruit less astringent and more palatable. Ripe fruits are used fresh as drupes, marmalades and jams. The ripe fruits are available from July to October and are sold for cheap price. The tree is popular in villages for its herbal uses and delicious fruits. Although little work on tannins, proteins and vitamin contents of fruits was reported[2-4], no systematic chemical investigation was done before. Therefore, we undertook a systematic chemical analysis of the air-dried ripe fruit instead of fresh ripe fruit as the living cells in fresh fruit cause trouble during exhaustive extraction of chemicals with organic solvents. We report here the results of our findings.

The ripe fruits of *F. jangomas* were collected from Agartala Market in July, 1987. The fruits were made into pieces and air-dried in mild sunlight between 30-36°C for 10 hr and thrashed by a pestle. Air-dried thrashed fruits (50 g) were extracted with petroleum ether (b.p. 60-80°) in a soxhlet and the petroleum ether extract was distilled to a gummy oil (1.6 g). The physico-chemical characteristics of this oil were determined by usual methods : iodine value (Wij's/30 min), 55.5; saponification equivalent (0.5 N alcoholic KOH), 159.6. A part of the oil (1.0 g) was saponified with methanolic IN KOH (25 ml) for 2 hr in an atmosphere of nitrogen. The solvent methanol was removed under reduced pressure and the residue diluted with water. The unsaponifiable matter was recovered by extracting the aqueous layer with solvent ether. After removal of unsaponifiable matter, the aqueous layer was acidified with dilute HCl to pH 2-3 and extracted with solvent ether. The ether layer was washed, dried over anhydrous sodium sulphate and evaporated to get an oil of fatty acids (0.4 g). The fatty acids mixture was methylated with diazomethane and methyl esters mixture was purified on silica gel column. The purified esters mixture was analysed on a pye Unicam 104 gas chromatograph equipped with a FID and a 10 per cent DEGS column. It was identified[6] as mixture of methyl esters in per cent of α-linolenic (44.5), palmitic (31.3), oleic (8.0), stearic (5.8), linoleic (4.8), hexadecadienoic (3.3) and some minor unidentified acids (2.3) by co-gas chromatography with authentic samples. The presence of unsaturated acid esters was also confirmed by GC analysis of hydrogenated product (10 per cent) of a part of methyl esters mixture under identical condition when the peaks of unsaturated esters were not found and the relative intensities of saturated esters were increased.

The defatted fruits residue was extracted with 90% ethanol in a soxhlet and the alcoholic extract was reduced to half and refluxed with conc HCl (5 ml) for 1 hr *in vacuo* to ensure hydrolysis of proteins. The resultant extract was neutralised and concentrated under reduced pressure to a residue (5 g). A part of the residue (1.5 g) was chromatographed[7,8] through Amberlite IR 120(H^+) and elution of column with 2N aqueous HCl afforded an eluate which on co-paper chromatography (co-pc)[9] with authentic amino acids revealed the presence of glycine, alanine, methionine, proline and hydroxyproline in it. Another part of the residue (1.5g) on similar chromatography through Amberlite IRA 400(OH^-) and elution of column with 2N aqueous acetic acid gave an eluate which was identified as a mixture of alanine and valine by co-pc with authentic amino acids. All these amino acids from their mixture were isolated as ninhydrin complexes by preparative-pc[10] and estimated colorimetrically[11] in μg per g of air-dried fruits as proline (8.0), hydroxyproline (3.19) methionine (1.17), alanine (0.53), glycine (0.53) and valine (0.31).

TABLE 1. CHEMICAL COMPOSITION OF AIR–DRIED RIPE FRUITS OF *FLACOURTIA JANGOMAS* (PANIALA)

Parameters	
Moisture (%)	23.05
Total ash (%)	3.46
Water insoluble ash (%)	0.78
pH	5.75
Titrable acidity (ml of 0.1N NaOH/g)	3.2
Total reducing sugars (%)	4.2
Fats (%)	3.2
Tartaric acid (%)	0.44
Proteins (%)	0.38
Phenolics	0.30
Potassium (mg/g)	12.56
Nitrogen (mg/100 g)	113.30
Total iron (mg/100 g)	18.37
Ferrous iron (mg/100 g)	11.81
Phosphorus (mg/100 g)	16.28
Ascorbic acid (mg/100 g)	2.12
Proline (μg/g)	8.80
Hydroxyproline (μg/g)	3.19
Methionine (μg/g)	1.17
Alanine (μg/g)	0.53
Glycine (μg/g)	0.53
Valine (μg/g)	0.3
Unidentified (tannins, pigments, sterols, polysaccharides, vitamins, fibres, etc.) (%)	63.5 (approx).

A little of air-dried fruits was accurately weighed in a pre-weighed crucible and was heated in an air oven at 110°C for 12 hr, cooled and weighed. From the difference in weight of the crucible, the moisture content of fruits was estimated (Table 1).

A known quantity of air-dried fruits in a pre-weighed crucible was strongly heated in a muffle furnace at 550°C for 6 hr, cooled and weighed to get the weight of the total ash (Table 1). The ash was shaken well with a little distilled water, centrifuged in a pre-weighed glass tube and the supernatant liquid was removed by decantation. The residue of the tube was dried in an oven at 110°C for 6 hr, cooled and weighed to get the weight of the insoluble ash (Table 1).

Air-dried fruits (15 g) were extracted with distilled water (150 ml) by warming on boiling water bath for 15 min and cooled to room temperature overnight. The aqueous extract was filtered and the filtrate was used for detection and determination of simple reducing sugars, pH, potassium, non-volatile organic acids, tartaric acid, nitrogen containing compounds, proteins, total as well as ferrous iron, phosphorus and ascorbic acid (Table 1). The presence of sugars in the filtrate was detected by Molisch's test[12] and of keto hexose by Selivanoff's test[12] and with $SnCl_2$, H_2SO_4 and urea[13]. The reducing sugars were estimated after making the aqueous filtrate free from proteins by passing through resin column and treating the eluate with anthrone reagent using glucose as standard. The protein free filtrate on co-pc[14] with authentic sugars showed the presence of arabinise, glucose, galactose and fructose. This was also confirmed by reducing a part of concentrated protein-free filtrate with $NaBH_4$ and then acetylating the resultant alditols mixture with acetic anhydride and pyridine followed by co-pc with standard alditol acetates. The pH of the aqueous filtrate was observed by a digital (Systronics) PH meter. The amount of non-volatile organic acids was estimated by titration with a standard 0.1 N NaOH solution. The presence of tartaric acid in the filtrate was detected by resorcinol test[15] and its quantitative amount was estimated by preceipitation as potassium hydrogen tartrate by a modified technique[16]. The presence of nitrogen containing compounds in the filtrate was detected by Dragendroff's reagent and nitrogen per cent was determined by Kjeldahl method[17]. Protein was detected by biuret test. The proteins were precipitated[9] from the filtrate by making alkaline with NaOH and followed by addition of 0.1 M solution of heavy metals (Cu^{++}, Pb^{++}, Hg^{++}). The precipitate was filtered, dried and proteins were estimated by Kjeldahl method[18]. The total as well as ferrous iron in the filtrate were determined separately by 1,10-phenanthroline reagent[19] using standard Mohr's salt solution as reference. Phosphorus in the filtrate as soluble phosphate was estimated with vanado-molybdate reagent by the method of Donald *et al*[16,20] using standard KH_2PO_4 solution as reference. Ascorbic acid (vitamin-C) in the filtrate was measured by the 2,6-dichlorophenol indophenol method[9]. Potassium in the filtrate was determined by flame photometry[19] using standard KCl solution as reference.

A part of the air-dried fruits (5g) was extracted with methanol and the methanolic extract was concentrated to a gummy residue. The residue was dissolved in n-butanol and extracted the phenolics with 5 per cent aqueous NaOH solution[16]. The aqueous layer was separated and was acidified with dilute HCl and extracted the free phenols separately with solvent ether and chloroform. Both the ether and chloroform layers were washed with water, dried over anhydrous sodium sulphate, mixed together and evaporated to dryness. The weight of the residue gave the amount of phenolics present in the fruits (Table 1).

All these data suggest the good nutritive value of the air-dried ripe fruits and to a certain extent these fruits may be used as a substitute for citrus fruits.

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References

1. Deb D B, *Flora of Tripura State*, Today & Tomorrow, New Delhi, 1981, 1, 232.
2. *The Wealth of India*, CSIR, New Delhi, 1952, 4, 42.
3. Malek A, Begum R and Ahmad K, Analysis of some foods of East Pakistan, *Pakistan J. Biol. agric. Sci.*, 1966, 9, 25.
4. Mannan A and Ahmad K, Studies on vitamin E in foods of East Pakistan, *Pakistan J. Biol. agric. Sci.*, 1966, 9, 13.
5. Dinda B and Guha S, Fatty acid composition of *Spilanthes paniculata*, *J. Indian Chem. Soc.*, 1988, 65, 146.
6. Christie W W, *Lipid Analysis*, Pergamon Press, New York, 1982, 2nd Edn., 63.

7. Lederer E. and Lederer M. *Chromatography*, Elsevier, New York, 1957, 127.
8. Stahl E, *Thin-layer chromatography*, Toppan, Tokyo, 1969, 2nd Edn. 737.
9. Plummer D T, *An Introduction to Practical Biochemistry*, Tata Mc Graw-Hill, New Delhi, 1979. 2nd Edn.
10. Abbott D. and Andrews R S, *An Introduction to Chromatography*, Houghton-Mifflin, Boston, 1965, 9.
11. Dinda B and Saha M K, Free amino acids of *Melastoma melabathricum*, *J Indian Chem Soc*, 1985, 62, 789.
12. Trease G E and Evans W C, *Pharamacognosy*, English Language Book Society/Bailliere-Tindall, Oxford, 1985, 12th Edn., 344.
13. Feigl F, *Spot Tests in Organic Analysis*, Elsevier, Amsterdam, 1975, 341. 352.
14. Dinda B and Saha M K, A Flavonol diglycoside from *Melastoma melabathricum*, *J Indian Chem. Soc.*, 1988, 65, 209.
15. Svehla G, *Text Book of Macro and Semimicro Qualitative Inorganic Analysis*, Orient Longman, New Delhi, 1982, 5th Edn. 373.
16. Garratt D C, *The quantitative Analysis of Drugs*, Toppan, Tokyo, 1964, 3rd Edn, 532, 632.
17. Vogel A I, *Elementary Practical Organic Chemistry*, Part-3, Longman, London, 1971, 652.
18. Mckenzie H A and Wahace H S, The Kjeldahl's determination of nitrogen, *Aust J Chem.*, 1954, 7, 55.
19. Bassett J, Denney R C, Jeffery G H and Mendham J, *Vogel's Text Book of Quantitative Inorganic Analysis*, English Language Book Society, Longman, 1985, 4th Edn, 742, 837.
20. Donald R, Schwehr E W. and Wilson H N, A rapid colorimetric method for the determination of phosphate, *J Sci Fd Agric*, 1956, 7, 677.

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MICROBIOLOGICAL STATUS OF GROUNDNUT OIL IN PUNE MARKET

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Microbiological status of groundnut oil samples, purchased from local market in Pune, was studied with special reference to lipolytic and pathogenic micro-organisms. Among the 26 oil samples examined, 83 per cent of unrefined groundnut, 100% ration and 43% refined groundnut oil samples were contaminated with microorganisms. The predominant pathogenic organisms were *Aspergillus flavus, Staphylococcus aureus* and *Bacillus cereus* which were also lipolytic in nature. Other lipolytic organisms were *A. niger*, *Penicillium* sp. and *Micrococcus luteus*.

Groundnut oil is commonly used as edible oil in Maharashtra. Apart from using oil as a frying medium, it is a common practice in Maharashtra to apply unheated groundnut oil to chapati before and after roasting. Unheated oil is also consumed alongwith powders of condiments, spices and also with chutney. Therefore, microflora of oil is important from the point of view of health of human beings and spoilage of oil. Although microflora of groundnut seeds has been reported, no information is available on microflora of groundnut oil. This paper presents microbiological status of groundnut oil collected from Pune market.

Twenty six oil samples comprising 12 unrefined groundnut, seven ration oil samples and seven refined groundnut oil samples (100 g each) sold loose except for three packed refined samples were procured from the local market and ration counters in Pune City.

Aliquots of the oil samples were diluted tenfold using sterilized quarter strength Ringer solution containing 0.1 per cent agar to stabilize the emulsion[1]. Enumeration of micro-organisms was done using 0.1 ml of suitable tenfold dilutions of oil emulsion made in quarter strength Ringer solution and the selective agar media by pour plate technique. For total viable count of bacteria, and yeasts and moulds, standard plate count agar[1] and Davis yeast extract salt agar[1] respectively were used. Enumeration of lipolytic bacteria, and yeasts and moulds was done using nutrient agar and Davis yeast extract salt agar respectively containing 10 per cent (v/v) sterilized groundnut oil (as lipid source) dyed with 0.04 per cent (w/v) nile blue sulphate[2]. For enumeration of food poisoning organisms selective media used were Mannitol salt agar[3] for *S. aureus*, Mannitol egg yolk agar[4] for *B. cereus* and *Aspergillus* differential medium[5] for *A. flavus*. The plates were incubated at room temperature (28 ± 2°C) for 48 hr for bacteria and 96 hr for yeasts and moulds. The number of colonies appearing on the plates and showing yellow colouration in case of *S. aureus*, yellow colouration with the formation of clear zone around the colony in case of *B. cereus*, orange-yellow colouration in case of *A. flavus* and blue colouration or precipitation around them in case of lipolytic organisms were counted. Coliforms were estimated by inoculating 10 ml, 1.0 ml and 0.1 ml of 10 per cent emulsion of oil samples in quarter strength Ringer solution with 0.1 per cent agar in MacConkey broth according to Most Probable Number (MPN) technique[6].

For isolations of *Salmonella* and *Shigella* group of organisms, 5.0 ml aliquots of undiluted oil samples were enriched in 45 ml selenite F broth at 37°C for 24 hr and isolations were made from enriched broths using Wilson and Blair agar and MacConkey agar[6] by streak plate method. The plates were incubated at 37°C for 48 hr. For isolation of lipolytic bacteria and yeasts and moulds, a loopful of the undiluted oil sample was streaked on nutrient agar and Davis yeast extract salt agar respectively containing 10 per cent (v/v) sterilized groundnut oil (as lipid source) dyed with nile blue sulphate. The plates were incubated at room temperature (28 ± 2°C) for 96 hr. Colonies showing lipolytic activity as evidenced by the blue colouration or precipitation around them were identified.

Bacterial isolates were identified according to Bergey's Manual[7] and yeasts according to Gibbs and Shapton[8] and Lodder[9]. Moulds were identified by microscopic observations of their wet mounts in lactophenol cotton blue stain and according to Barnett[10] and Kamat[11].

Microbiological examination with respect to total viable count of the groundnut oil samples under study showed 83 per cent contamination in unrefined groundnut oil; 100 per cent in ration oil and 43 per cent in refined groundnut oil as detailed in Table 1.

Microbial profile of these oil samples as illustrated in Fig 1 shows that all the samples were free from coliform organisms. In case of unrefined groundnut oil, the total viable count for bacteria ranged between 0.0 and 1.8×10^4 cfu/ml oil, yeasts and moulds between 0.0 and 3.6×10^4, lipolytic bacteria between 0.0 and 0.6×10^4, lipolytic yeasts and

TABLE 1. INCIDENCE OF MICROBIAL CONTAMINATION IN GROUNDNUT OIL SAMPLES EXAMINED

Oil examined		No. of samples contaminated	% contamination
Type	Number		
Unrefined	12	10	83
Ration	7	7	100
Refined	7	3	43

TABLE 2. SAMPLEWISE OCCURRENCE OF LIPOLYTIC MICROBIAL SPECIES

Sample No.	Bacteria	Yeasts and moulds	Total
	Unrefined groundnut oil		
1	*Bacillus licheniformis*	*Aspergillus niger*	2
2	*Bacillus licheniformis* *Pseudomonas alcaligenes* *Bacillus subtilis*	*Aspergillus flavus* *Aspergillus niger* *Penicillium sp.*	6
3	------	------	
4	*Klebsiella pneumoniae*	*Aspergillus flavus*	2
5	*Micrococcus luteus*	*Penicillium sp.*	2
6	*Micrococcus cryophilus* *Pseudomonas mendocina*	*Aspergillus niger* *Aspergillus flavus*	4
7	*Bacillus cereus* *Staphylococcus aureus*	*Aspergillus niger*	3
8	------	------	0
9	------	------	0
10	------	------	0
11	------	------	0
12	------	------	0
	Ration shop oil samples		
13	*Micrococcus luteus* *Staphylococcus aureus* *Bacillus licheniformis*	*Aspergillus flavus*	4
14	*Bacillus subtilis* *Bacillus cereus*	*Hansenula silvicola*	3
15	*Klebsiella pneumoniae* *Micrococcus luteus*	*Aspergillus flavus* *Rhizopus* sp.	4
16	*Staphylococcus aureus* *Micrococcus luteus* *Bacillus cereus*	*Penicillium* sp.	4
17	*Bacillus subtilis* *Citrobacter freundii*	*Aspergillus niger*	3
18	------	*Penicillium* sp. *Aspergillus flavus* *Hansenula silvicola*	3
19	*Bacillus cereus* *Citrobacter freundii*	*Penicillium* sp.	3
	Refined oil		
20	------	------	0
21	------	*Aspergillus niger* *Pencilillium* sp.	2
22	------	------	0
23	------	------	0
24	------	*Aspergillus niger*	1
25	------	------	0
26	*Staphylococcus aureus*	*Penicillium* sp.	2

TABLE 3 INCIDENCE OF LIPOLYTIC MICROBIAL SPECIES IN GROUNDNUT OIL SAMPLES

Microorganisms isolated	Oil samples examined				Total % incidence
	Unrefined (12)	Ration (7)	Refined (7)	Total (26)	
Aspergillus niger	4 (33)	2 (29)	2 (29)	8	31
Penicillium sp.	2 (17)	3 (43)	2 (29)	7	27
Aspergillus flavus	3 (25)	3 (43)	0	6	23
Bacillus cereus	1 (8)	3 (43)	0	4	15
Micrococcus luteus	1 (8)	3 (43)	0	4	15
Staphylococcus aureus	1 (8)	2 (29)	1 (14)	4	15
Bacillus licheni formis	2 (17)	1 (14)	0	3	12
Bacillus subtilis	1 (8)	2 (29)	0	3	12
Citrobacter freundii	0	2 (29)	0	2	18
Hansenula silvicola	0	2 (29)	0	2	8
Klebsiella pneumoniae	1 (8)	1 (14)	0	2	8
Micrococcus cryophilus	1 (8)	0	0	1	4
Pseudomonas alcaligenes	1 (8)	0	0	1	4
Pseudomonas mendocina	1 (8)	0	0	1	4
Rhizopus sp.	0	1 (14)	0	1	4

Figures in parenthesis in column headings indicate number of samples analysed.
Figures in parenthesis represent per cent incidence of microbial species in that particular group of oil.

moulds between 0.0 and 0.4 $\times$ 10^4, *S. aureus* and *B. cereus* between 0.0 and 0.1 $\times$ 10^4 and *A. flavus* between 0.0 and 0.2 $\times$ 10^4 cfu/ml oil. The total viable count in ration oil samples ranged from 0.1 $\times$ 10^4 to 1.5 $\times$ 10^4 cfu/ml oil for bacteria, 0.1 $\times$ 10^4 to 4.5 $\times$ 10^4 for yeasts and moulds. 0.0 to 1.0 $\times$ 10^4 for lipolytic bacteria, 0.1 $\times$ 10^4 to 1.4 $\times$ 10^4 for lipolytic yeasts and moulds, 0.0 to 0.3 $\times$ 10^4 for *S. aureus* and *A. flavus* and 0.0 to 0.4 $\times$ 10^4 for *B. cereus*. The refined oil samples were free from *B. cereus* and *A. flavus* although the total viable count for bacteria ranged between 0.0 and 0.4 $\times$ 10^4 cfu/ml oil, for yeasts and moulds between 0.0 and 0.3 $\times$ 10^4, for lipolytic bacteria, lipolytic yeasts and moulds and *S. aureus* varied from 0.0 to 0.2 $\times$ 10^4 cfu/ml oil.

The identification of the lipolytic isolates (Table 2) revealed the following:

Bacillus cereus, B. licheniformis, B. subtilis, Citrobacter freundii, Klebsiella pneumoniae, Micrococcus cryophilus, M. luteus, Pseudomonas alcaligenes, P. mendocina, Staphylococcus aureus, Hansenula silvicola, Aspergillus flavus, A. niger, Penicillium sp. and *Rhizopus* sp. The possible source of these organisms could be the oilseeds, soil, oil storage containers and handling methods.

Enteric pathogens viz. *Salmonella* and Shigella group were absent in all the samples examined.

Total incidence (Table 3) of *A. niger* was maximum viz. 31 per cent followed by *Penicillium* sp. (27 per cent), *A. flavus* (23 per cent), *S. aureus, B. cereus* and *M. luteus* (15 per cent) and *B. subtilis* and *B. licheniformis* (12 per cent).

The higher incidence in unrefined and ration oils is alarming since these oils are consumed by a large section of the population. Presence of *A. flavus, S. aureus* and *B. cereus* in the oil samples examined indicates potential cause of food poisoning while presence of lipolytic species shows possibility of spoilage (lipolysis) in food products where the unheated oil is used.

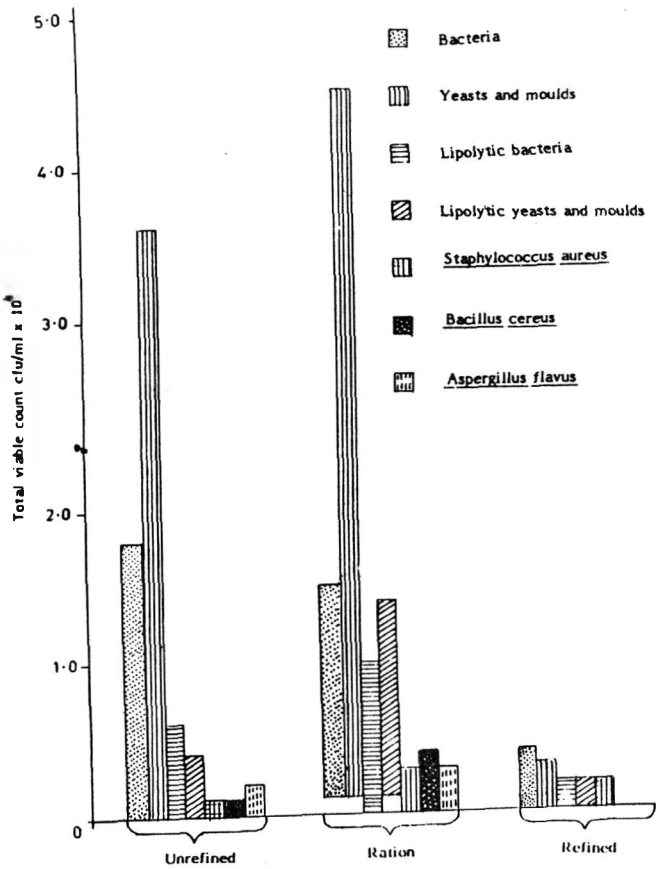

Fig.1 Microbial profile of groundnut oil samples.

References

1. Harrigan W F and McCance M E, *Laboratory Methods in Food and Dairy Microbiology*, Academic Press, London, 1976, 208.
2. Skerman V B D. *Detection of lipolytic organisms. In A Guide to the Identification of the Genera of Bacteria* Williams and Wilkins Co., Baltimore, 1967, 2nd Edn., 242.
3. Corry J E L Roberts D and Skinner F A, *Isolation and Identification Methods for Food Poisoning Organisms* Academic Press, New York, 1982, 165.
4. Mossel D A A, Koopman M J and Jongerius E, Enumeration of *Bacillus cereus* in foods *Appl. Microbiol.* 1967, 15, 650.
5. Bothast R J and Fennell D I, A medium for rapid identification and enumeration of *Aspergillus flavus* and related organisms, *Mycologia*, 1974, 66, 365.
6. Cruickshank R Duguid J P and Swain R H A, *Medical Microbiology*, English Language Book Society, E and S Livingstone Ltd. Edinburgh 1965, 11th Edn.
7. Buchnan R E and Gibbons N E, In *Bergey's Manual of Determinative Bacteriology*. Williams and Wilkins Co., Baltimore, 8th Edn. 1974.
8. Gibbs B M and Shapton D A, *Identification Methods for Microbiologists*, part B, Academic Press, London, 1968, 151.
9. Lodder J The Yeasts — *A Taxonomic Study*. North Holland Publishing Co., London, 1970, 2nd Edn. 226.
10. Barnett H L, *Illustrated Genera of Imperfect Fungi*. Burgess Publishing Co., Minn., U.S.A., 1956, 2nd Edn, 62.
11. Kamat M N, *Hand Book of Mycology, Part I, Phycomycetes and Ascomycetes*, Prakash Publishing Co., Poona, 1959.

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STUDIES ON THE INCIDENCE OF *VIBRIO CHOLERAE* IN FISHERY PRODUCTS

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The incidence of *V. cholerae* in fishery products collected from Kerala and Tamil Nadu coasts during 1986-87 is reported. *V. cholerae* 01 was present to the extent of 0.2% of raw fishery products, whereas *V. cholerae non 01* was present in 26.3% of raw and 12.14% of frozen products.

During the period from 1st Feb. 1986 to 30th June 1987, 1734 samples of marine products comprising shrimp, cuttle fish, squid, lobster, clam, finfish and mussel were tested. The samples were drawn from different fish processing units, primary process centres, landing places and fish markets situated at Calicut, Cochin, Quilon, Vizhinjam, Tuticorin, Mandapam and Madras. These sample collection spots are along the coastline of Kerala and Tamil Nadu which together contributed 49 per cent of the total marine products export from India. Similarly, 52 per cent (1981 to '86) of the total rejections due to *Vibrio cholerae* was also from these two States - 9 tons from Kerala and 42 tons from Tamil Nadu.

The samples taken in sterile dishes were separately wrapped in polythene paper and transported to the laboratory under aseptic conditions by keeping them in sufficient quantity of ice taken in an insulated box. The analysis was normally started within an hour of collecting the samples. The samples were tested for *V. cholerae* as follows. Twenty five grams of the samples were enriched in 225 ml alkaline peptone water (APW) for 16-18 hr at 37°C and one 3 mm loopful from the surface was streaked on to TCBS agar plate. Also 1 ml from the first enrichment was transferred to 9 ml APW and after incubation at 37°C for 6-8 hr, one loopful was streaked on to another TCBS agar plate. The suspected colonies from TCBS agar plates after 18-24 hr incubation at 37°C were confirmed by the biochemical and serological tests as per the method carried out by Varma *et al*[1].

The results of the study indicated that *Vibrio cholerae* 01 was present in only 2 out of 1001 samples of fresh fish analysed. The first isolation was from fresh whole shrimp collected from a retail fish market at Cochin and the second from fresh cuttle fish fillet that had undergone pre-processing at Tuticorin. On serotyping, the former was identified as "Ogawa" and the latter as "Inaba". *V. cholerae 01* was absent in all the samples of frozen fishery products, which may be due to the better hygienic and sanitary conditions prevailing

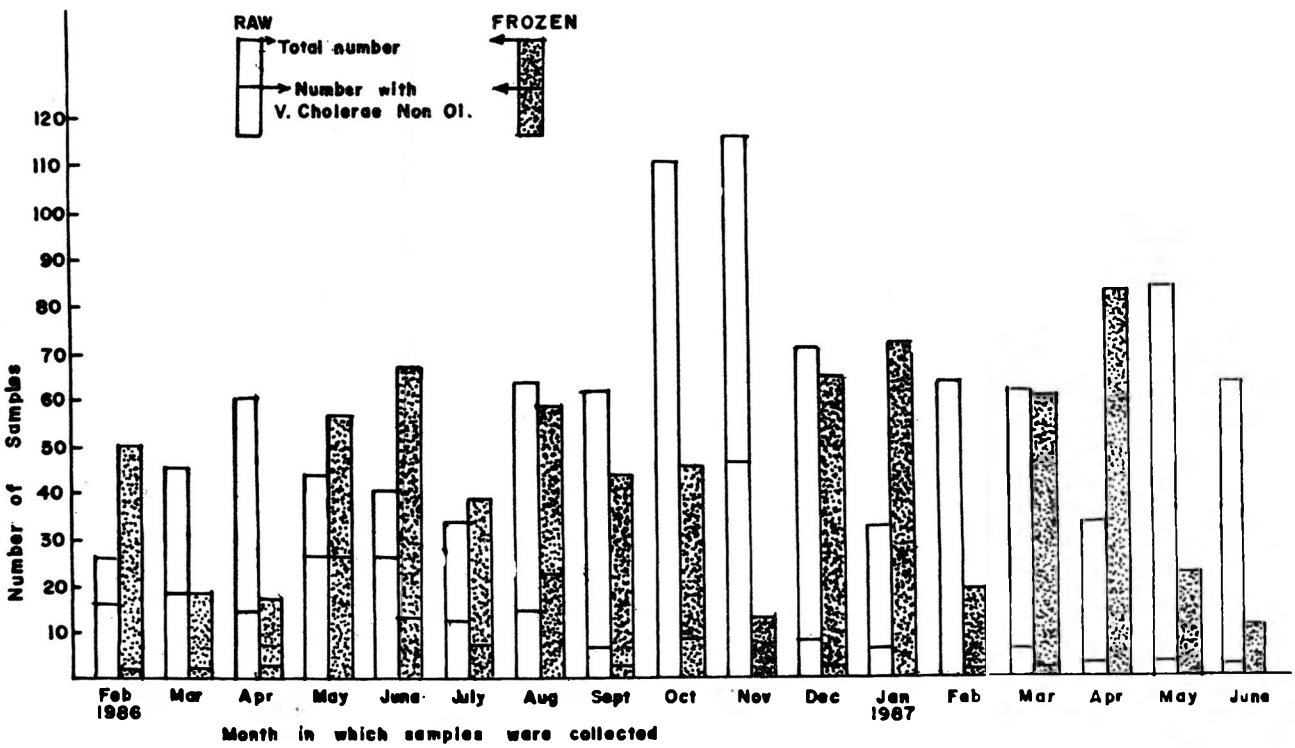

Fig.1. Incidence of *V. cholerae* non 01. from Feb. 1986 to June 1987

* Research scheme on *V. cholerae*. C/o CIFT, Cochin - 682 029

TABLE 1 INCIDENCE OF *V. CHOLERAE* NON-01 IN FISH

Sample	No. of samples examined		No. of samples showing incidence of V. *cholerae* non 01	
	Raw	Frozen	Raw	Frozen
Shrimp	728	287	194 (26.6)	34 (11.8)
Cuttle fish	23	4	7 (30.4)	0
Squid	46	44	6 (13.0)	5 (11.3)
Lobster	13	160	3 (23.0)	30 (18.7)
Clam	31	95	11 (35.4)	17 (17.8)
Finfish	85	143	32 (37.6)	3 (2.0)
Mussel	75	—	10 (13.3)	—
Total	1001	733	263 (26.3)	89 (12.14)

Figures in parentheses are the percentages.

in the processing units compared to the pre-processing units and markets.

Incidence of *Vibrio cholerae* non 01 was noted in all categories of samples except frozen cuttle fish as given in Table 1. Although the detection of *Vibrio cholerae* non 01 has not been recognised as one of the criteria for rejection of consignments by the Fish Inspection Laboratories, there are a few references showing the pathogenicity of this organism[2,3].

There was a marked variation in the percentage of incidence of *Vibrio cholerae* non 01 between unfrozen (raw) and frozen samples and also between different categories of products. In all the cases, significantly low level of incidence can be seen in frozen samples compared to the corresponding unfrozen (raw) samples. Finfish has shown the highest percentage (37.6) of incidence of *Vibrio cholerae* non 01 among unfrozen samples. De *et al*[4]. have also reported a similar observation on the incidence (38 per cent) of *Vibrio cholerae* non 01 in marine fish samples collected from Calcutta Region. Squid samples showed the lowest percentage of incidence of *Vibrio cholerae* non 01 amount unfrozen samples.

The difference in the pattern of the percentage of incidence of *Vibrio cholerae* non 01 between fresh (raw) and frozen samples during the course of the study has been illustrated in Fig.1. A marked decline can be observed in the percentage of incidence as the study progressed. This can be attributed to the advice and feed-back given by the study team to the concerned people soon after the analysis and during their next visit for sample collection.

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References

1. Varma, P R G, Gopalakrishna Iyer T S and Nair M R, Vibrio cholerae in seafoods and processing environments. A preliminary study, *Export Insp. J.* 1986, 2, 28.
2. Aldova E, Lazniekova K, Stepankova E and Lietowa J, Isolation of non-agglutinable vibrios from an enteritidis outbreak in Czechoslovakia, *J. Infect Dis*, 1968, **118**, 25.
3. Angelo De Paola, Vibrio cholerae in Marine Foods and Environmental waters - A literature review, *J Fd Sci*, 1981, **46**, 66.
4. De S P, Banerjee M, Deb B C, Sengupta P G, Sib J, Sircar B K, Sen D, Ghosh A, and Pal S C, Distribution of vibrios in Calcutta environment with particular reference to *V. parahaemolyticus*, *Indian J med, Res*, 1977, 65, 21.

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UTILISATION OF DIFFERENT FOOD AND AGRICULTURAL WASTES FOR THE PRODUCTION OF THE YEAST *HANSENULA ANOMALA* VAR *SCHNEGGII*

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The optimum C:N ratio for growth of *Hansenula anomala* var. *schnegii* in a complex medium was determined. Different food and agricultural wastes used for growth of the yeast were analysed to determine their carbohydrate and nitrogen contents. Of the different wastes tested, pineapple waste extract, tomato pomace extract and soy whey allowed max. cell growth (12-12.8 g/l) while the protein content of the cell was max (40-41% of dry cell wt) when the organism was grown in a medium containing rice and wheat bran extract.

Food processing wastes and waste water which were earlier discarded are now being used as substrate for the production of yeast biomass. Citrus peel juice[1], coffee berry wastes[2], whey[3], soy whey[4], coconut waste water[5], date carbohydrate[6], grape pomace[7], tapioca starch waste water[8] and brewery wastes[9] have been successfully used for growth of yeast. Acid-treated corn cobs[10], rape seed oil meal[11], enzymatically treated potato wastes[12] and rice straw[13] have also been used as low cost substrates for growth of various yeasts.

The present study shows the optimum C:N ratio in a complex medium and the effect of different agricultural and food processing wastes on growth of the yeast *Hansenula anomala* var *schnegii*.

Hansenula anomala var *schneggii* was maintained on modified Czapek-Dox medium[14]. For development of inoculum, the yeast cells were transferred from the agar slants to 50 ml of medium consisting of glucose 1, urea 0.1, KH_2PO_4 0.1, $MgSO_47H_2O$ 0.5, per cent, $FeSO_47H_2O$ 1 p.p.m, $MnSO_44H_2O$ 1 p.p.m, pH 4.5 taken in a 250 ml conical flask. This was placed on a rotary shaker (150 rpm) at 28°C. After 72 hr of growth, 0.2 ml (10^7 cells) of the cell suspension was used as inoculum.

The basal medium used for investigating the C:N ratio in a complex medium contained KH_2PO_4 0.1 per cent and $MgSO_47H_2O$ 0.05 per cent (pH 4.5). To this, the carbon and organic nitrogen sources each were added at 1, 1.5 and 2 per cent levels. While determining the cell yield in waste liquor the requisite C:N ratio was maintained by supplementing the medium with ethyl alcohol or urea when necessary. The salts KH_2PO_4 (0.1 per cent) and $MgSO_47H_2O$ (0.05 per cent) were also added, the initial pH being 4.5. In a typical experiment 50 ml of the medium was taken in 250 ml conical flask and inoculated with 0.2 ml yeast cell suspension. The flasks were placed on a rotary shaker (150 rpm) for 72 hr at a temperature of 28 $\pm$ 1°C. After fermentation, the cells were harvested by centrifuging at 3000 rpm, washed once with normal saline and then dried at 60°C for 24 hr to constant weight. The cell yield was expressed as grams per litre. The final pH of the broth was determined by a glass electrode.

The nitrogen content of the wastes and the yeast cell was determined by the micro-kjeldahl method and the crude protein content (N×6.25) of the dry cell was then calculated. The carbohydrate content of the wastes was calculated in terms of glucose by standard Fehlings method. The hydrolysed extracts of groundnut cake, coconut cake, mustard cake and mango kernel after clarification were directly estimated for their reducing sugar content (as glucose) by Fehlings method. The other wastes were first hydrolysed with 1N H_2SO_4 by refluxing in a water bath for 7 to 8 hr. The hydrolysates were then neutralised with NaOH clarified with 20 per cent $ZnSO_4$ solution and glucose content measured by standard Fehlings solution.

Peels, cores and other rejected portions of mango, pineapple, tomato and potato were collected from local food processing plants. Wheat and rice bran were also collected. The extracts were made by heating the wastes with water in an autoclave at 121°C at 15 lb pressure for 15 min followed by filtration. The solid content of the filtrate was determined. Groundnut, coconut and mustard cakes were obtained from the market and made free from any remaining traces of oil by solvent extraction. The fibrous coating of the mango stone was removed and the kernel taken out. This was soaked in water to remove toxic substances dried and crushed. A 10 per cent (W/V) aqueous slurry was hydrolysed by 1N H_2SO_4 by heating at 121°C in an autoclave for 15 min followed by filtration. The solid content of the filtrate was determined. Soy whey was obtained by precipitating the protein from soy milk with 1 per cent (W/V) acetic acid. Soy flour was brought from the market in a dry form. Distillary waste was collected from a local distillery and molasses obtained from the local market.

The carbon to nitrogen ratio for optimum biomass yield was determined in a complex medium. Previous studies on the effect of carbon and nitrogen sources on cell growth showed the superiority of glucose and ethyl alcohol as the carbon source and cornsteep liquor and urea as the nitrogen source (Mukerjee and Majumdar, unpublished work). Of the different simple and complex nitrogen sources tested, cornsteep liquor allowed maximum cell growth. Therefore, the optimum C:N ratio was determined using cornsteep liquor as the source of organic nitrogen and ethyl alcohol and glucose as the source of carbon. Results indicated that maximum cell

TABLE 1 CARBOHYDRATE, NITROGEN AND SOLID CONTENT OF DIFFERENT WASTE MATERIALS USED FOR GROWTH OF *HANSENULA ANOMALA* VAR *SCHNEGGII*

Wastes	Nitrogen (%)	Carbohydrate (as dextrose) (%)	Solid content (W/V) (%)
Mango waste extract	0.02	3.34	4.00
Pineapple waste extract	0.04	2.40	5.50
Tomato pomace extract	0.05	0.26	2.10
Groundnut cake extract	0.26	0.43	2.30
Coconut cake extract	0.17	1.20	2.80
Mustard cake extract	0.11	0.53	2.40
Mango kernel extract	0.05	1.47	3.02
Potato waste extract	0.01	0.43	2.00
Wheat bran extract	0.06	0.83	1.75
Rice bran extract	0.06	0.63	1.97
Soy whey	0.01	0.50	4.20
Distillery waste	0.25	1.65	7.90
Molasses	1.13	50.80	60.00
Soyflour	6.70	35.80	—

TABLE 2. EFFECT OF DIFFERENT WASTE MATERIALS ON GROWTH OF *HANSENULA ANOMALA* VAR *SCHNEGGII*

Wastes	Cell growth (g/l)	Final pH of booth	Protein (% of dry cell wt)
Pineapple waste extract	12.8	5.1	28.75
Tomato pomace extract	12.0	4.4	30.60
Soy whey	12.0	5.0	30.00
Coconut cake extract	8.8	5.1	36.80
Groundnut cake extract	8.5	4.6	31.60
Rice bran extract	8.5	2.8	41.20
Wheat bran extract	8.8	3.1	40.75

growth was recorded in a medium containing 2 per cent ethyl alcohol and 1.5 per cent cornsteep liquor with a C:N ratio of 23:1.5.

A large number of wastes available locally were tested for their effect on yeast growth. As the feasibility of utilising these wastes would depend on their composition, the carbohydrate (as glucose) nitrogen and solid contents were determined (Table 1). After supplementation with a carbon source i.e. ethyl alcohol or nitrogen source i.e. urea (a superior simple organic nitrogen source) when necessary, the wastes were used as substrate for growth of the yeast *Hansenula anomala* var *schnegii* in the requisite C:N ratio of 23:1.5. This contained 1.054 g of carbon (accounting for the carbon in the total sugar of cornsteep liquor) to 0.069 g of nitrogen. Of the different wastes tested (Table 2), pineapple waste extract, tomato pomace extract and soy whey allowed maximum cell growth (12-12.8 g/l). Coconut cake extract, groundnut cake extract, rice and wheat bran extract also allowed abundant growth (8.5-8.8 g/l). Mustard cake extract, mango kernel extract, potato waste extract, soyflour, distillery waste and molasses gave lower yields of cell biomass (2.8-6.8 g/l) (Results not presented). The greater amount of growth in pineapple and tomato pomace extract and soywhey may be due to proper balance of nutrients viz sugars, organic nitrogen compounds and minerals in these wastes. In those cases where sufficient cell growth was recorded i.e. 8 g/l or above, the crude protein content of the yeast cells was determined. Maximum protein content (40-41 per cent of the dry cell wt) was recorded in the medium containing rice and wheat bran extract.

References

1. Matales R T, *Single Cell Protein*, S R, Tannenbaum and DIC Wang (Eds) MIT Press, Cambridge, Mass, 1975, II, 208.
2. Fries KWE, U.S. Patent 3,576—720, 1971.
3. Muller L L, Yeast products from whey, *Proc Biochem* 1969, 4,21.
4. Kobayashi T, Yamanoi A and Otsuka S I, U.S. Patent, 3974, 030, 1976.
5. Smith M E and Bull A T, Protein and other compositional analysis of *Saccharomyces fragilis* grown on coconut waste water, *J appl Bacteriol*, 1976, 41, 97.
6. Kamel B S, Utilisation of date carbohydrate as substrate in microbial fermentation, *Proc Biochem*, 1976, 14, 12.
7. Kail S Z, Dudkin M S and Skornyakova N S. The use of grape pomace to obtain food yeast, *Izv Vyssh Ucheben Zaved Pisch Tekhonol*, 1971, 5, 92.
8. Than N C and Wu J S, Treatment of tapioca starch waste water by Torula yeast, *Can, Inst. Fd Sci Technol J*, 1975, 8, 202.
9. Shanon L J and Stevenson K E, Growth of *Calvatia gigantea* and *Candida Steatolytica* in brewery wastes for microbial protein production and BOD reduction, *J Food Sci*, 1975, 40, 830.
10. Nagy Gyula, Milagros Vaillant, Klara Balint and Anik Sos, Fermentation of agricultural wastes by yeasts, *Biotechnol Bioengg* 1975, 17, 1823.
11. Garg S K Sharma S and Sandhu S K, Repeaseed oil meal as substrate for single cell protein production. *Indian J exp Biol*, 1983, 21, 297.
12. Moreton R S, Growth of *Candida utilis* on enzymatically hydrolysed potato waste, *J appl Bacteriol*, 1983, 21, 297.
13. Araiyo A and D'Souza J, Enzymatic saccharification of pretreated rice straw and biomass production, *Biotechnol Bioengg*, 1986, 28, 1503.
14. Thom C and Raper K B, *A Manual of Aspergilli*, The Williams & Wilkins Co., Baltimore, 1945, 39.

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OPTIMISING THE PARAMETERS FOR PEARLING SORGHUM

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Pearling of sorghum was carried out with different types of rollers like continuous emery stone, conical emery stone, conical concrete, conical granite and intermittent emery stone in a TNAU Model Pearler, At ¼" clearance and 1250 r.p.m. the bran removal of continuous emery stone roller was 105 g per 1000 g of sorghum. Addition of 5% moisture and conditioning time of 4 hr have enhanced the pearling.

Sorghum continues to be used as a whole meal flour by the poorer sections of the people all over India especially in dry tracts but it is quite unpopular among those accustomed to softer cereals like rice and wheat. The palatability of the sorghum could be improved by a pearling process which removes a part of bran and germ. Rice milling machinery with various abrasive devices used in conventional grain milling were tried for continuous, large scale pearling of sorghum[1]. Hence, the present investigation was carried out to identify an equipment to achieve a pearling efficiency of around 90 per cent after giving pretreatments to grain.

TNAU Model Pearler was used for pearling studies. The pearler is of abrasive type consisting of a cylinderical metalic box with perforations at the bottom, roller and a 1 H.P. electric motor. A screw conveyor is fitted at the end of the roller to facilitate the easy removal of the grain from the box. The diameter and length of the abrasive roller are 14 in. and 6 in. respectively. The pearler was fitted with a variable speed gear unit with the following specifications input speed 1500 r.p.m. output speed 325-2200 r.p.m. The experiments were carried out at different speeds viz., 750, 1000, 1250 r.p.m. The clearance between the roller and sieve adjusted was ¼ in., ½ in. and ¾ in. Five different abrasive rollers namely 1) continuous emery stone rollers 2) Intermittent emery stone roller (having gap between rollers) 3) conical emery stone roller 4) conical concrete roller 5) conical granite roller were tried and their performances were studied with Co 24 variety of the sorghum.

The feed rate of the sorghum that is to be passed through the pearler was standardised by operating at different feed rates (2,3,4 and 5 kg/hr). The amount of water to be added which enhances the removal of bran was optimized by adding water (30, 40, 50, 60 and 70 ml/kg). The amount of bran removed, the yield of the pearled sorghum and breakage were estimated. The conditioning time after the addition of water to sorghum grains was also standardized with different conditioning times (2, 3, 4, 5 and 6 hr). For these experiments, water was added to the sorghum and the samples were kept in closed containers without any loss of moisture. Three replications were maintained for all the experiments.

The effect of different rollers on pearling of sorghum at various speed and clearance is presented in Tables 1, 2 and 3. From Tables 1 and 2, it could be observed that the performance of the continuous emery stone roller is better than the other types of rollers in removing the bran from sorghum grains. The intermittent emery roller also showed good results. The conical emery stone roller, conical concrete roller, conical granite stone roller were very poor in pearling of sorghum as can be seen from the amount of bran removed. The efficiency of these rollers is 50 per cent lesser than that of continuous emery stone roller. This is mainly due to the less abrasive area available for the rollers to pearl the sorghum. As the clearance between roller and sieve increased, the efficiency of pearler decreased. At ½ in. clearance and 1250 r.p.m. the bran removal of continuous emery stone roller was 71 g where as at ½ in. clearance and 1250 r.p.m., the bran removal in continuous emery stone roller was 86 g. At low r.p.m. of 750 and 1000, the efficiency of pearler was 10.5 to 16 per cent more when the clearance is ¼ in. The data also indicated that as the speed of the rollers increased, the

TABLE 1. EFFECT OF DIFFERENT ROLLERS ON PEARLING OF SORGHUM AT VARIOUS SPEEDS

Type of roller	Speed (rpm)	Pearled sorghum (g)	Bran removed (g)	Tr. value
Continuous emery stone	750	957 (8.8)	43	6.48*
"	1000	937 (9.1)	63	7.93*
"	1250	929 (10.2)	71	8.36*
Conical emery stone	750	984 (4.8)	16	3.98*
"	1000	977 (5.3)	23	4.78*
"	1250	966 (6.1)	34	5.82*
Conical concrete	750	984 (4.1)	16	3.98*
"	1000	978 (5.7)	22	4.68*
"	1250	964 (6.6)	36	6.02*
Conical granite stone	750	978 (4.3)	22	4.68*
"	1000	974 (5.2)	26	5.09*
"	1250	965 (6.7)	35	5.91*
Intermittant emery stone	750	960 (6.4)	40	6.32*
"	1000	943 (7.2)	37	6.07*
"	1250	937 (8.7)	63	7.90*

Wt of sorghum taken 1 kg : Clearance between roller and sieve ½ in.
CD (P=0.05) value for 750 rpm = 0.005
CD (P=0.05) value for 1000 rpm = 0.008
CD (P=0.05) value for 1250 rpm = 0.01
Figures in the parenthesis indicate the percentage of breakage
*Figures are transformed values (Tr. value)

TABLE 2. EFFECT OF DIFFERENT ROLLERS ON PEARLING OF SORGHUM AT VARIOUS SPEEDS

Type of roller	Speed (rpm)	Pearled sorghum (g)	Bran removed (g)	Tr. value*
Continuous emery stone	750	950 (9.8)	50	7.06
	1000	930 (10.0)	70	8.36
	1250	914 (11.6)	86	9.24
Conical emery stone	750	980 (5.7)	20	4.43
	1000	974 (6.2)	26	5.09
	1250	962 (7.4)	38	6.15
Conical concrete	750	978 (5.3)	22	4.65
	1000	976 (7.2)	24	4.89
	1250	960 (8.0)	40	6.32
Conical granite	750	974 (5.3)	26	5.02
	1000	971 (6.4)	29	5.37
	1250	958 (7.2)	42	6.47
Intermittant emery stone	750	950 (7.7)	50	7.06
	1000	936 (8.3)	64	7.99
	1250	920 (8.9)	80	8.92

Wt of sorghum taken 1 kg : Clearance between roller and sieve ¼ in.
CD (P=0.05) value for 750 rpm = 0.01
CD (P=0.05) value for 1000 rpm = 0.078
CD (P=0.05) value for 1250 rpm = 0.024

Figures in the parenthesis indicate the percentage of breakage
*Figures are transformed values

pearling performance also increased. At 1250 r.p.m. the pearling efficiency was 72-90 per cent more than at 750 r.p.m. for different rollers. However, the vibrations were more at 1500 r.p.m. and the pearler could not be run at this speed. Since the performance at ¾" clearance was very poor (Table 3) with continuous emery stone roller further trials were not carried out with other types of rollers at this clearance. It was reported that 12 per cent pearling was achieved for sorghum when the clearance was kept at 2.00 mm in a runner type disc sheller. The breakage loss varied from 8.8 to 11.6 per cent in continuous emery stone roller and 5.3 to 8 per cent in other rollers.

Addition of 2-3 per cent water enhances the removal of bran sorghum as reported by Desikachar[3]. It is reported that a

TABLE 3. PEARLING OF SORGHUM WITH CONTINUOUS EMERY ROLLER

Speed (rpm)	Pearled sorghum (g)	Bran removed (g)
750	973 (12.2)	27
1000	954 (13.2)	46
1250	948 (14.7)	58

Wt of sorghum taken 1 kg; Clearance between roller and sieve 1.95 cm.

commercial Dandekar type rice mill satisfactorily removed bran from sorghum moistened with 3-5 per cent water and conditioned for 5 to 15 min[2]. The experiments showed the removal of bran from sorghum to 92 g. The addition of more water (60 ml or 70 ml/kg) did not show any increase in bran removal of bran from sorghum to 92g. The addition of more the sieve and caused problems to clean the pearler. The bran removal was only 75 and 88 g with addition of 30 and 40 ml/kg of water. It is observed from the trials that the conditioning time of 4 hr was the optimum period for the moisture to move into the endosperm of the sorghum grain in loosening the bran from the other portions of the grain. Conditioning time of 4 hr with 5 per cent moisture increased the bran removal from 92 to 105 g. More conditioning time of 5 and 6 hr did not facilitate the bran removal. The removal of bran with less conditioning time of 2 and 3 hr was only 85 and 92 g. The study revealed that the pearling of sorghum in a TNAU Model Pearler is maximum of 105 g per 1000 g at 0.65 cm. clearance at 1250 r.p.m. Addition of 5 per cent moisture and conditioning time of 4 hr have enhanced the pearling by 18 per cent.

References

1. Viraktamath C S, Raghavendra G, and Desikachar H S R, Use of rice milling machinery for commercial pearling of grain sorghum (Jowar) and culinary uses for pearled sorghum products, *J Fd Sci Technol*, 1971, 6, 11.
2. Sahay K M and Gandhi A P, Studies on the pearling and milling of sorghum, *J Fd Technol*, 1985, 20, 245.
3. Desikachar H S R, Pearling and milling studies on sorghum grain quality. *Proceedings of the International Symposium on Sorghum Grain Quality*, ICRISAT Center, Patancheru, India, Oct. 1981, 194.

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STUDIES ON MECHANICAL DRYING OF COPRA

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Experiments were conducted in a laboratory model thin layer drying set-up for the determination of drying characteristics of copra at air flow rates of 19.57, 38.37 and 82.88 m^3/h and drying air temperatures of 50, 65, 80 and 95°C. For reducing the moisture content of copra from 50% (w.b) to 7% (w.b) it took only 20 hr of drying in mechanical drying at an optimum temperature of 65°C as compared to the duration of 7-10 days by sun-drying.

Copra, the dried kernel of coconut is the richest source of vegetable oil, containing 65-70 per cent oil. The nuts are converted into copra by drying from about 55 per cent (w.b) moisture content to about 7 per cent (w.b) for oil extraction. This oil is used extensively for edible and industrial purposes and the cake is a valuable feed for cattles. Various drying methods such as sun-drying and primitive kilns are used. However by the traditional method of sun-drying, 7-9 days are taken for complete drying which requires more drying area and labour, besides resulting in losses by birds and rodents. Hence, this investigation was carried out to study the drying characteristics of copra by mechanical drying.

In the studies, matured tall variety coconuts obtained from the University farms were used. To study the drying characteristics of copra, laboratory model dryer shown in Fig.1. was used. It consists mainly of a centrifugal blower, powered by a 2 hp three phase electric motor, rotating at 2880 r.p.m., electrical heaters of 1.5 KW capacity each (3 numbers) and a metallic holding bin. The drying air temperature and flow rate were varied by using a thermostat and by varying the speed of the blower with step pulley arrangement respectively. The flow rate of hot air was calculated from the velocity of flow and the area of cross section of the air flowing section. Three to five coconuts were split and dried during the experiments. The drying experiments were conducted at 19.57, 38.37 and 82.88 m^3/hr air flow rates at 50, 65, 80 and 95°C hot air temperatures. After drying to about 35 per cent (w.b) moisture content of kernel and shell, scooping of kernel or meat from the shell was done. The drying was continued at all air flow rates and temperatures to reach a final moisture content of 6-7 per cent (w.b). The weights lost during the experiments were recorded at two hour intervals. Moisture content determinations were done as per the AOAC Method, by drying 5 g of sample at 130 ± 1°C for 1 hr^1. Also, drying experiment was performed by sun-drying and the moisture contents were determined by recording the losses in weight at four hour intervals.

The effect of hot air temperature and air flow rate on the drying time of copra is presented in Fig.2. It is seen from Fig.2, that the drying time is reduced with the increase in the hot air temperature and air flow rate. The time taken

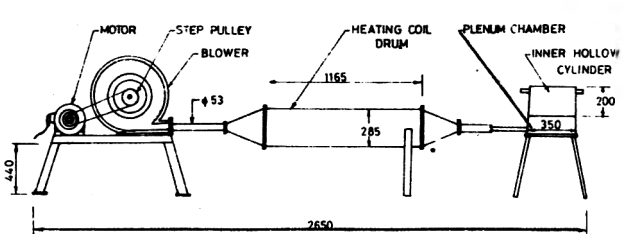

Fig. 1. Laboratory model thin layer dryer

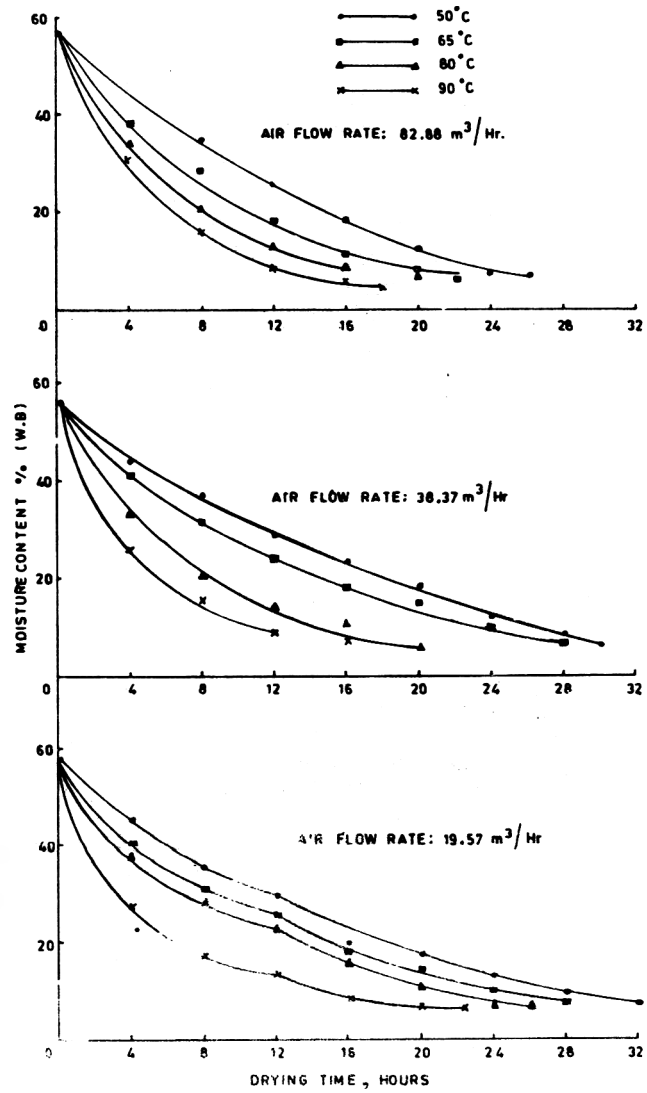

Fig.2 Effect of temperature and air flow rate on drying copra.

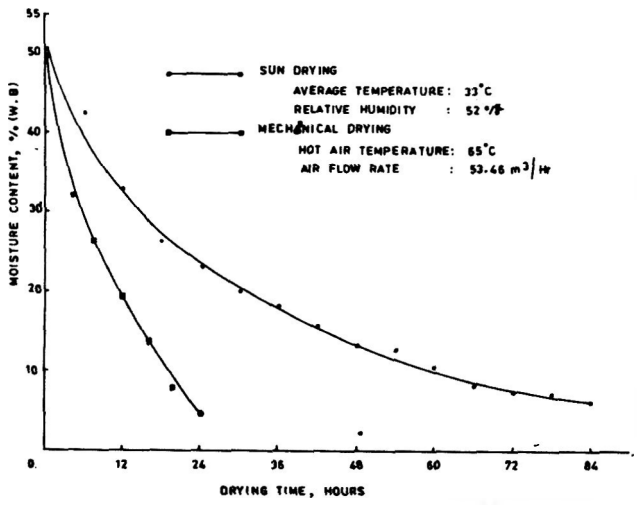

Fig.3. Drying characteristics of copra by mechanical and sun-drying methods.

for drying coconut from 50 per cent (w.b) to 6 per cent (w.b) moisture content at 5°C and 19.5 m^3/hr of air flow rate is 33 hr. For the same reduction of moisture content, the time taken for drying is 19 hr at 95°C at the same air flow rate. The time required for drying coconut at 38.37 m^3/hr air flow rate, 50°C and 95°C temperature are 30 and 15 hr respectively. Similarly, when the air flow rate is 82.88 m^3/hr, the drying times for the same moisture reduction are 23 and 15 hr for hot air temperatures of 50 and 80°C respectively.

The experimental results have shown positive non-linear correlation of drying time on drying air temperature and air flow rate. Also, it was observed that the product dried at 80°C and 95°C hot air temperatures resulted in hard and discolouration of the copra which are deterimental to the oil quality. Hence, the drying air temperature of 65°C was found to be optimum in mechanical drying. The drying times found are in agreement with the ones reported by other research workers[2].

Under large scale trial, about 2,500 nuts were dried in the mechanical drier with the agricultural waste-fuelled furnace. The average hot air temperature was adjusted to 65°C at 53.46 m^3/h air flow rate with coconut shell as fuel. It took 21 hr of drying to reduce the moisture content from 50 per cent (w.b) to 7 per cent (Fig. 3). For the same reduction in moisture content under sun-drying, the duration was 76 sunshine hours which could be obtained in about 8-10 days (Fig.3). The copra dried in the mechanical dryer will yield upto 65 per cent of oil of good quality as the drying is done under hygienic environment.

References

1. *Official Methods of Analysis,* Association of Agricultural Chemists, Washington, 1961.
2. Palmer J A, Some aspects of copra-drying. Cocoa and coconuts in Malaya. *Proceedings of the Symposium in Kualalampur,* September, 1967.

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COMPOSITION OF INDIAN SOYABEAN LECITHIN

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Lecithin from indigenous soya oil was separated into neutral lipids, glycolipids and phospholipids, which were then further resolved into individual components and estimated. Fatty acid composition of commercial lecithin as well as of the fractions was also determined. Analytical characteristics and composition agreed with those of U.S. lecithin.

Present production of soybean oil (*Glycine Max.*) in India is around 280,000 MT[1], indicating to a potential of around 32,000 MT of soya lecithin (1.8 per cent basis.) At present lecithin used in India is still imported and an investigation on indigenous soya lecithin appeared worth pursuing. Accordingly, a study of the gross characteristics, of the nature of Indian soya lecithin and the amounts of the different components such as neutral, glycolipids, phospholipids was undertaken.

Crude soybean oil was extracted in the laboratory from soybean seeds collected from Pantnagar, U.P. by solvent extraction method. The clarified oil was heated to 50-60°C, hot water at 95°C added in amounts equal to the phosphatide content of about 2 per cent (v/v) and stirred continuously, at 250-300 r.p.m. for 10-15 min at that temperature. Hydrated phosphatides were removed by centrifugation in a Remi Centrifuge (R23) of the oil-water mixture at 2000 r.p.m. (RCFX g=700) for 10 min. The lipids of the crude lecithin were separated into the three classes by silicic acid column chromatography[2]. The silicic acid (BDH, column chromatography grade, particle size 60—120 mesh) was washed with water and methanol, dried, and activated at 110°C overnight before use. The column was eluted in the order (i) chloroform (175 ml) for neutral lipids, acetone (700 ml) for glycolipids and methanol for phospholipids (175 ml). Different eluates were desolventised and vacuum dried. Percentage of each class was determined by gravimetry as 31.0, 4.9 and 64.1 per cent respectively (cf. U.S. lecithin 36.8, 13.2, 50.0). Neutral lipid class obtained was further fractionated into different components by preparative TLC on silica G (thickness 0.5 mm) using solvent system petroleum-ether (40-60°) diethyl ether-acetic acid (80:20:1). The lipids were visualised by spraying with 0.1 per cent 2'-7' dichloroflorescein soln in ethanol and viewing under UV light. Bands were identified by comparing the R_f value with standard samples spotted along-side, triglycerides, free fatty acids and diglycerides with diethyl ether: methanol (90:10), followed by chloroform: methanol: water (50:45:5)[3]. Eluates were evaporated after filtration, and vacuum dried. The residue on weighing afforded 95, 1.5, and 3.4 per cent respectively of these class of compounds. Glycolipids were separated by preparative TLC on silica gel G with solvent system chloroform — methanol — 28 per cent ammonia (65:25:5). Different fractions were visualised, with iodine vapour, identified by comparing the R_f with literature, extracted with appropriate solvent systems, and estimated gravimetrically after vacuum drying. Steryl glycoside ester, monogalactosyl diglyceride, digalactosyl diglycerides were estimated to be present in 35, 16.9 and 48.1 per cent respectively.

The phospholipid group was further resolved into individual phospholipids by preparative TLC on silica gel G (0.5 mm thickness) using chloroform-methanol-water (65:25;4). Spots were visualised using various spray reagents[3]. The phospholipids were identified from response to spray reagents and by comparing R_f values with literature data. For gravimetric estimation, the phospholipids were separated in a similar manner, visualised with iodine and extracted using solvent system chloroform-methanol (2:1). The extracts obtained was filtered, desolventised and each individual phospholipid was estimated gravimetrically. Phosphatidyl choline, phosphatidyl ethanol amine, phosphatidyl inositol, phosphatidyl glycerol, lysolecithin, phosphatidic acid were present in 34.8, 23.6, 33.8, 4.5, 2.8 and 1.5 per cent respectively.

Methyl esters of the fatty acids of different lipid classes were prepared by treatment of lipids with boron trifluoride-methanol[3]. Fatty acid methyl esters were analysed in Varian 3700 Gas Liquid Chromatograph with flame ionisation detector. A 6 ft by ⅛ inch stainless steel column packed with 3 per cent EGSS supported on Aeropak 30 (80/100) was used. Oven temperature was 160°C Injector and FID temperatures were 200° and 230°C respectively. Carrier gas (N_2) flow-rate was 25 ml/min. The weight percentage of different fatty acids was calculated with a computer data system CDS 111.

Acetone soluble and benzene insoluble materials, moisture and acid value were determined by published methods[4]. Colour was determined by Lovibond Tintometer using a ¼" cell after dilution with benzene. Viscosity of the lecithin samples were determined using a Brookefield Viscometer. Colour and viscosity was compared with those of an imported soyalecithin sample.

In order to compare the emulsifying property of Indian soya lecithin, with that of imported lecithin, the following experiment was done: 90 gm of hydrogenated fat, 5 mg Annato dye, and 10 gm of the lecithin sample under study were blended thoroughly. 6.5 gm of lecithin fat blend was added to 100 ml. of hot water (70-80°) and stirred in a Remi electrical stirrer for about one minute. A 100:1. aliquot of the resultant water-lecithin fat blend was poured into a 100

TABLE 1. FATTY ACID COMPOSITION OF DIFFERENT LIPID CLASSES OF INDIAN SOYA LECITHIN

	Fatty acid composition (wt.%)				
Lipid class	16:0	18:0	18:1	18:2	18:3
Lecithin, commercial	10.8	3.9	24.2	54.8	6.3
Neutral lipid	8.5	3.8	32.1	50.6	5.0
Glycolipid	20.5	3.7	17.1	47.8	10.9
Phospholipid	11.4	3.7	20.6	57.5	6.8

ml graduated cylinder and allowed to stand at room temperature for one hour. In both cases, the water layer was less than one ml. which indicated comparable emulsion stability.

Fatty acid composition of commercial soybean lecithin (Table 1) indicates that linoleic acid was the major fatty acid present (54.8 per cent), the amount of linolenic, oleic, stearic and palmitic acid being 6.3, 24.2, 3.9 and 10.8 per cent respectively (cf. data obtained by Weber on American Soya Lecithin[3]-linolenic 6.8, oleic 17.7, stearic 4.0, palmitic 17.4). A high linoleic content, followed by oleic, palmitic, linolenic and stearic, were observed in all the lipid classes. The neutral lipid class, essentially triglyceride, shows only 5 per cent linolenic acid (cf 6.8 per cent in U.S. lecithin). This is to be expected, since India-grown soybean oil contains 3.7-6.6 per cent linolenic acid compared to 8-11 per cent in American varieties. Palmitic and linolenic acid proportions of the glycolipid fraction were higher, and linoleic acid proportion lower, compared to the total lecithin. In case of phospholipid, the palmitic and linoleic acids were slightly higher and oleic acids lower than the total lecithin. Similar trends have been observed for the corresponding classes in U.S. lecithin also.

TABLE 2. CHARACTERISTICS OF INDIAN SOYBEAN LECITHIN

Parameter	Indian soybean	U.S. soya lecithin specification
Acetone-insoluble material (wt.%)	6.0	62 (min)
Benzene-insoluble material (wt.%)	0.1	0.3 (max)
Moisture (%)	0.5	1 (max)
Acid value (as % oleic acid)	27.0	30 (max)
Viscosity (poises at 25°C)	110.0	150 (max)
Colour	Comparable to imported lecithin	10 (max) on Gardner Scale

Identity and composition of different lipid classes present in Indian soya lecithin under investigation agree closely with the literature data available on U.S. lecithin. The physical properties of Indian soya lecithin, particularly its emulsifying properties, can therefore be expected to be of the same order as for U.S. lecithin. The analytical characteristics (Table 2) satisfy U.S. specifications for soya lecithin, and colour and viscosity also match those of imported lecithin. The slightly lower level of linolenic acid could result in slightly greater resistance to autoxidation and development of off-flavour in Indian soya lecithin.

The valuable phosphatide containing gummy materials obtained during degumming crude soybean oil in India ought to be upgraded to standard quality lecithin which can meet food and non-food applications and avoid imports.

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References

1. 44th All India Convention of Oil Technologists' Association of India 11th — 12th Feb. 1989.
2. Kates, M. *Techniques of Lipidology*, North Holland Publ Co, Amsterdam, 1975, 400, 552, 372, 437.
3. Weber, E J, Compositions of commercial corn and soybean lecithins, *J. Am Oil Chem Soc*, 1981, 58, 898.
4. Cocks, L V, and Rede, C Van, *Hand Book of Oils and Fats Analysis*, Academic Press, Inc., New York, 1966, 137.

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EFFECT OF PRETREATMENTS ON THE QUALITY OF SWEET TURNIP PICKLE

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An investigation undertaken to study the effect of various pretreatments of ingredients on the quality of the sweet turnip pickle revealed that, (i) steam blanching is better than water blanching and dry roasting of slices of turnips, (ii) sugar is superior to refined jaggery (shakkar) and jaggery, (iii) gingelly oil is as good as mustard oil for preparation of pickle and (iv) roasting of spice imparted better quality to the pickle as compared to frying and steam sterilisation.

In Indian pickles, a large variety of spices and condiments are used in their preparation besides edible oils. The condiments not only impart particular flavour and taste to pickles but also act as mild disinfectants on account of their essential oil components. The keeping quality of sweet turnip pickle is not more than 3-4 months with the present day method of preparing it as described in CFTRI leaflet[1]. The composition of spices and their ingredients may not only be responsible for enhancing non-enzymatic browning but also a source of microbial contamination[2]. Therefore, an investigation was undertaken to study the effect of various pretreatments on the quality of the sweet turnip pickle.

The turnips (variety 'Purple Top' White Globe'), spices and condiments were obtained from the local market. The pickle was prepared as per the method suggested in CFTRI leaflet. The turnip roots free from damages, bruises and spoilage were selected and washed in clean water. The slices of 0.5 cm thickness prepared from them on a slicing machine were blanched in boiling water for 5 min, cooled in running cold water and drained on a perforated tray. Onion, ginger and garlic were made into fine pulp in a mixer and fried in half the quantity of required oil, till the pulp attained a brownish colour. The mustard, black pepper, cinnamon and cumin (white) were coarsely powdered in a mixer to which the required quantity of salt and powdered chillies were added. The sliced turnips were mixed with the fried material and spices and filled into glass jars.To this, acetic acid and a thick extract of tamarind were added. After storing for 5 days, a thick syrup of jaggery was added and finally on 10th day the remaining half of the quantity of oil was added and packed in glass containers.

The above method was modified with the following alterations in different pretreatments:

i) Water-blanched turnips were compared with steam-blanched and dry-roasted ones.
ii) All the ingredients except the vinegar were steam-sterilized in an autoclave for 20 min at 15 lb/in^2 to kill the surface flora.
iii) The spices were roasted with and without mustard oil and added to the pickle.
iv) Mustard oil was boiled and added to the pickle.
v) Mustard oil was replaced with gingelly oil.
vi) Jaggery was replaced with refined jaggery (shakkar) and sugar.

All the pre-treated materials were stored at ambient temperature (8.2-33.9°C) and the analyses were carried out at monthly intervals for sensory quality[3], acid value[4], peroxide value[4] and non-enzymatic browning (NEB)[5].

Data presented in Table 1 show that the dry roasting of turnips caused maximum browning of pickle and an increase in acid value. Steam-blanching was found to be the best among all the three treatments with respect to better acceptability, lesser NEB and acid values.

It is also evident from Table 1 that jaggery is one of the major constituents responsible, causing early spoilage in pickle. Its addition increased browning, the acid and peroxide values of the oil. The addition of refined jaggery was found to be better as compared to crude jaggery for retention of quality.

The browning due to addition of jaggery may probably be ascribed to the presence of impurities in jaggery like caramalized sugar and various metallic ions such as iron which can form complex with tannins finally imparting an undesirable colour to the product. This was further confirmed from the results by the substitution of jaggery with refined jaggery and sugar. With refined jaggery, the product looked better and with sugar the quality further improved and similar trends in quality were maintained during the storage also.

The acid and peroxide values were also found to be higher in pickles with jaggery as compared to refined jaggery and sugar. This may be due to the fact that the impurities in jaggery such as metallic ions cause degradation of oil during storage.

From the above, it is clear that the use of sugar instead of jaggery would improve the quality of the pickle during storage and also reduce the degradation of oil in the pickle.

The data regarding the effect of oil on quality of the pickle show that the pickle without oil was organoleptically acceptable and as good as the pickle with boiled mustard oil However, the boiling of mustard oil improved the organoleptic quality of the pickle over the unboiled one. This treatment is similar to the one in other pickles where the pickle is kept in sun after preparation.

However, the pickle prepared without oil (except little oil used for frying spices) was as good as that prepared with

TABLE 1. EFFECT OF PRETREATMENTS ON THE QUALITY OF SWEET TURNIP PICKLE

Treatment	Storage period (months)	Colour	Flavour	Texture	Overall	NEB (O.D. at 420 nm)	Acid value (mg/g)	Peroxide value (milli-moles/kg)
Control (water-blanching, jaggery, mustard oil)	0	6.8	7.0	7.0	6.9			
	2	6.5	6.3	6.3	6.4			
	4	5.6	5.2	5.3	5.4	0.42	22.40	3.80
Dry-roasting	0	7.0	6.8	7.3	7.0			
	2	5.5	6.2	6.2	5.9			
	4	5.0	5.0	5.3	5.1	0.51	21.28	3.50
Steam-blanching	0	7.0	7.1	7.2	7.1			
	2	5.6	6.1	6.2	6.0			
	4	5.4	5.5	5.6	5.5	0.39	18.00	3.40
Refined jaggery	0	7.3	7.2	7.2	7.2			
	2	7.0	6.3	6.3	6.5			
	4	5.4	6.2	5.6	5.7	0.33	21.28	3.70
Sugar	0	7.3	6.8	7.2	7.1			
	2	6.6	6.3	6.5	6.5			
	4	6.4	5.9	5.6	6.0	0.27	12.32	3.50
Without oil	0	7.1	7.2	7.0	7.1			
	2	6.6	6.3	6.2	6.4			
	4	6.0	5.0	5.0	5.3	0.52	31.36	4.00
Boiled mustard oil	0	7.2	7.0	7.5	7.2			
	2	6.6	6.0	6.5	6.4			
	4	5.7	5.2	5.2	5.4	0.31	27.36	5.00
Gingelly oil	0	7.3	6.8	7.5	7.2			
	2	6.5	6.2	6.5	6.4			
	4	5.8	5.0	5.2	5.3	0.43	22.40	4.00
Steam sterilized spices	0	6.5	7.0	6.7	6.7			
	2	6.0	6.0	6.0	6.0			
	4	5.6	5.0	5.4	5.3	0.29	18.40	4.50
Roasted spices	0	7.3	6.7	7.2	7.0			
	2	6.3	6.3	6.6	6.4			
	4	6.0	5.4	5.3	5.6	0.26	18.96	3.50
Spices fried in oil	0	7.0	6.6	7.0	6.9			
	2	6.5	6.5	6.3	6.4			
	4	6.3	5.8	6.3	6.1	0.29	19.52	3.25

oil but browning was found to be maximum. This may be due to the absence of a covering film of oil among the slices and between the pickle and head space inside the container. Since, only little quantity of oil was used for frying the spices it resulted in formation of more free fatty acids and increase in peroxide value on heating. Similar observations have been recorded by Sultana and Sen[6] where they have shown an increase in free fatty acids and peroxide value on heating of safflower and groundnut oil.

Since, there was not much difference in the quality of the pickle prepared with mustard oil and gingely oil, the gingely oil could form a good substitute to the mustard oil.

Data presented in Table 1 also indicate that the steam-sterilization was not good as roasting and frying of spices. This could be attributed to the removal of volatile compounds from spices during steaming. The pickle in this case took longer time for curing.

Roasting of the spices improved the quality of the pickle with regard to the organoleptic quality and NEB. However, peroxide value and acid value were little high and pickle was

acceptable even upto 4 months. The improvement of organoleptic quality on roasting may be because of various intermediary compounds formed during roasting of spices. Vasundara[7] reported that about 24 compounds of allyl isothiocyanate derivatives were formed during roasting of mustard which contributed to the development of flavour.

Similarly, frying of spices has also been found to improve the organoleptic quality of pickle.

From the above discussion, it can be concluded that steam-blanching of turnip slices, use of sugar instead of jaggery and roasting of slice may be helpful in improving the quality of sweet turnip pickle. Gingelly oil can form as a substitute for mustard oil.

References

1. *Homescale Processing and Preservation of Fruits and Vegetables*. Central Food Technological Research Institute, Mysore, 1977,50.
2. Thangamani R, Mattada R, and Sankaran R, Microbial contamination in spices. *Indian Fd Pckr*, 1975, 29, 11.
3. Amerine M A, Pengborn R M, and Roessler E B, *Principles of Sensory Evaluation of Food*. Academic Press, New York, 1965.
4. Jacobs M B, *Chemistry and Technology of Foods and Food Products*, Inter Science Publishers, Inc., New York. 1958, vol. IV.
5. Ranganna S, *Manual of Analysis of Fruits and Vegetable Products*. Tata McGraw Hill Publishing Company Limited, New Delhi. 1977.
6. Sultana S N, and Sen D P, Studies on deep fat frying-changes during heating of oil, *J Fd Sci Technol*, 1979. 16, 208.
7. Vasundara T S Parihar B B, and Vijaya Raghavan, Flavouring compounds formed in roasted brown mustard (*Brassica juncea*), *Nahrung*, 1981. 25, 685.

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DIETARY FIBER CONTENTS OF SELECTED VEGETABLES AND FRUITS

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Dietary fiber contents (neutral detergent fiber and acid detergent fiber) are estimated in most commonly used vegetables (15) and fruits (8) of Indian origin. Edible portion, moisture and lignin content of above vegetables and fruits are also estimated for comparison. Among green leafy vegetables mint leaves (*Mentha spicata*) had highest neutral detergent fiber and acid detergent fiber, among other vegetables bitter gourd (*Momordica charantia*) had highest neutral detergent fiber and french beans had highest acid detergent fiber. Among the fruits guava (*Psidium guajava*) was rich both in neutral and acid detergent fibers.

In recent years, there has been a renewed interest in the dietary fiber content of foods and the physiological effect of its intake in man[1]. Dietary fiber is associated with many diseases of the Western world where the diets are high in refined carbohydrates and low in dietary fiber. Epidemiological studies have indicated that the low consumption of dietary fiber is related to diverticular diseases and other colon disorders[2]. Hence, dietary fiber has gained an important status in the field of human nutrition.

The method generally followed for the determination of carbohydrates in food is by difference, i.e. by deducting the sum of the measured moisture, ash, protein and fat from the total weight. The value obtained in this way changes by the determination of dietary fiber. The value of carbohydrates 'by difference' includes all types of carbohydrates from simple sugars to complex heteropolysaccharides, in addition to other substances such as organic acids and lignin[3]. Generally, food tables give the crude fiber content of foods, which is not the same as dietary fiber. For the determination of true dietary fiber values, three different methods are generally used. They are detergent methods of Van Soest[4,5], Southgate's method[3] and enzymatic method of Hellendoorn[6]. Values obtained by using three methods are higher than crude fiber values, hence closer to physiologically unavailable fiber.

There are very few studies which provide information of dietary fiber of tropical foods[7-10]. In the present study, the dietary fiber content and its related fractions are estimated for a variety of tropical fruits and vegetables and the results obtained are compared with other vegetables and fruits.

Vegetables used in the present investigation represented the classes of leafy vegetables, roots and tubers and other vegetables. For the purpose of comparison, the following vegetables were selected for the estimation of dietary fiber — amaranth greens (*Amaranthus gangeticus*), cabbage (*Brassica oleracea* var. *capitata*), chakotha greens (*Chenopodium album*), Chikki greens (*Rumex acetosella*), drumstick leaves (*Moringa oleifera*), kilkeerai greens (*Amaranthus tricolor*), mint leaves (*Mentha spicata*), spinach leaves (*Spinacia oleracea*), ash gourd (*Benincasa hispida*), bitter gourd (*Momordica charantia*), bottle gourd (*Lagenaria vulgaris*), French beans (*Phaseolus vulgaris*), pumpkin (*Cucurbita maxima*), beet root (*Beta vulgaris*), and carrot (*Daucus carota*).

The fruits were selected based on local availability and different types keeping in view of the consumption. They included apple (*Malus sylvestris*), banana (*Musa paradisiaca*), guava (*Psidium guajava*), green grapes (*Vitis vinifera*), orange (*Citrus aurantium*), papaya (*Carica papaya*), sweet lime (*Citrus sinensis*) and ripe tomato (*Lycopersicon esculentum*).

Both vegetables and fruits were procured fresh from the local market at five different times in one calender year and estimations were done in duplicate for each batch.

The selected fruits and vegetables were washed, cleaned and the edible portion was culled out and weighed. Depending upon the nature of vegetable, the samples were either dried in an oven at 40°C or freeze-dried and powdered. Vegetables with high moisture content as ash gourd and bottle gourd were freeze-dried using a Virtis freeze drier.

For sampling of fruits, the inedible portions were discarded, except in the case of guava and tomato the whole fruit was taken as edible. Some of these processing steps were based on the habits followed in eating the fruits in many tropical countries. The fruits were freeze-dried after taking a portion of the fresh sample for the estimation of the initial moisture content. The freeze-dried samples were stored over fused calcium chloride in a dessicator. Moisture content in the sample was determined by the standard AOAC method[11]. Methods developed by Van Soest were used for the determination of acid and neutral detergent fibers[4,5]. Acid detergent fiber (ADF) measures cellulose and lignin. Neutral detergent fiber (NDF) measures total cell wall material i.e. cellulose, hemicellulose and lignin. Hemicellulose is calculated by difference. Both the methods are used together to arrive at dietary fiber components.

Mean and standard deviation were calculated for all the experimental data by following the method described by Snedecor and Cochran[12].

The results of the present study i.e., edible portion, moisture, NDF, ADF, and lignin, are given in Tables 1 and 2. These values are on fresh weight basis. Leafy vegetables had low edible portion ranging from 58 to 78 per cent except cabbage which had 93 per cent edible portion. This is due to excessive amount of stems and roots present which are not

TABLE 1. DIETARY FIBER FRACTIONS IN SELECTED VEGETABLES*

Vegetable	Edible portion (%)	Moisture (%)	Neutral detergent fiber (%)	Acid detergent fiber (%)	Lignin (%)
Amarnath (*Amaranthus gangeticus*)	58	89.3 ± 0.88	2.45 ± 0.57	1.61 ± 0.31	0.48 ± 0.26
Cabbage (*Brassica oleracea* var. *capitata*)	93	93.4 ± 0.57	1.00 ± 0.19	0.82 ± 0.15	0.34 ± 0.11
Chakotha (*Chenopodium album*)	69	91.7 ± 1.05	1.33 ± 0.18	0.90 ± 0.35	0.29 ± 0.22
Chikki (*Rumex acetosella*)	50	93.5 ± 0.98	1.08 ± 0.22	0.78 ± 0.13	0.30 ± 0.12
Drumstick leaves (*Moringa oleifera*)	68	74.4 ± 1.53	2.56 ± 0.24	2.12 ± 0.47	0.75 ± 0.27
Kilkeerai (*Amaranthus tricolor*)	68	90.3 ± 1.11	2.01 ± 0.28	1.41 ± 0.31	0.39 ± 0.40
Mint (*Mentha spicata*)	78	87.5 ± 0.99	4.27 ± 0.42	4.00 ± 0.45	1.74 ± 0.30
Spinach (*Spinacia oleracea*)	66	94.2 ± 0.45	1.15 ± 0.15	0.68 ± 0.22	0.19 ± 0.14
Ash gourd (*Benincasa hispida*)	78	95.5 ± 0.24	0.78 ± 0.13	0.58 ± 0.16	0.10 ± 0.07
Bitter gourd (*Momordica charantia*)	80	90.62 ± 0.52	2.27 ± 0.23	1.47 ± 0.39	0.44 ± 0.37
Bottle gourd (*Lagenaria vulgaris*)	83	94.8 ± 0.35	0.79 ± 0.19	0.61 ± 0.23	0.09 ± 0.10
French beans (*Phaseolus vulgaris*)	90	90.5 ± 1.15	1.89 ± 0.23	1.51 ± 0.24	0.25 ± 0.06
Pumpkin (*Cucurbita maxima*)	76	89.6 ± 2.44	1.58 ± 0.40	1.07 ± 0.35	0.45 ± 0.35
Beet root (*Beta vulgaris*)	90	89.0 ± 0.83	1.34 ± 0.39	0.65 ± 0.15	0.22 ± 0.12
Carrot (*Daucus carota*)	87	86.4 ± 0.84	1.76 ± 0.28	1.40 ± 0.32	0.24 ± 0.07

*Values are mean ± SD of ten replicates and are on fresh wt basis.

edible. Other vegetables had comparatively higher edible portion ranging from 76 to 90 per cent. Green leafy vegetables had higher moisture content (86-94 per cent) than other vegetables (76-90 per cent). Ash gourd had the highest moisture content (96 per cent).

NDF values were correspondingly higher in all the samples than ADF and the values ranged from 1.0 to 4.2 g. Mint leaves had highest content of NDF (4.27 g), the lowest NDF was in ash gourd (0.78 g). All other vegetables had NDF in the range of 1 — 2.5 g on fresh weight basis. The ADF values also followed a similar trend as that of NDF being highest in mint leaves and lowest in ash gourd. Among dietary fiber fractions, mint leaves had highest lignin fraction. Lowest values, for lignin were obtained in bitter gourd. It may be noted here that dietary fiber fraction values which have been reported in literature[13] for some vegetables such as cabbage, spinach, bottle gourd, french beans and carrots are comparable to the values estimated in the present investigation. This gives relatively true picture of a mixture of tender, medium and mature vegetables that are available under any market condition and are likely to be consumed in everyday dietaries.

The edible portions of fruits were very high when compared to vegetables as shown in Table 2 and varied from nearly 70 per cent for orange and papaya to nearly 99 per cent for guava. Fruits had higher moisture content as compared to vegetables as evident from Tables 1 and 2. The NDF value and other dietary fiber fractions were highest in guava. This is due to

TABLE 2. DIETARY FIBER FRACTIONS IN SELECTED FRUITS*

Fruits	Edible portion (%)	Moisture (%)	Neutral detergent fiber (%)	Acid detergent fiber (%)	Lignin (%)
Apple (*Malus sylvestris*)	93	85.6 ± 3.61	1.02 ± 0.31	0.95 ± 0.20	0.32 ± 0.15
Banana (*Musa paradisiaca*)	68	76.7 ± 3.65	0.85 ± 0.28	0.54 ± 0.16	0.31 ± 0.12
Guava (*Psidium guajava*)	99	80.5 ± 0.82	5.16 ± 1.40	3.44 ± 1.39	1.47 ± 0.76
Green grapes (*Vitis vinifera*)	85	90.9 ± 0.80	1.46 ± 0.41	1.33 ± 0.37	0.54 ± 0.18
Orange (*Citrus aurantium*)	70	88.4 ± 0.58	0.92 ± 0.32	0.77 ± 0.28	0.12 ± 0.10
Papaya (*Carica papaya*)	70	91.6 ± 1.15	0.81 ± 0.26	0.72 ± 0.18	0.28 ± 0.18
Sweet lime (*Citrus sinensis*)	72	88.3 ± 1.79	1.55 ± 0.24	1.46 ± 0.19	0.58 ± 0.24
Tomato (ripe) (*Lycopersicon esculentum*)	100	93.6 ± 1.89	1.12 ± 0.32	0.96 ± 0.32	0.51 ± 0.23

*Values are mean ± SD of ten replicates and are on fresh wt basis.

the presence of seeds in guava which are many a times consumed with the fruit. Papaya had lowest NDF value of 0.81 g. The values of NDF for other fruits were between 0.8 and 1.6 g. Other dietary fiber fractions followed a similar trend. These values are comparable to the values reported in literature for dietary fiber fractions for certain fruits[10,13].

Since NDF values are more closer to physiologically unavailable carbohydrates, the values reported in this investigation can be used for planning of diets incorporating fruits and vegetables. They are useful for planning high/low fiber diets. The role of dietary fiber in slow release of sugar and in turn its role in management of diabetics is well documented[14-16].

Although customary Indian diets are high in dietary fiber and a large portion of it comes from cereals, others like vegetables and fruits contribute significantly towards dietary fiber intake. The information presented in this paper can be useful for nutritionists in computing unavailable carbohydrates in diets. Some of these inexpensive fruits and vegetables with their bland flavour could well form a good supplement to the low fiber diets as well.

References

1. Kelsay J L, A review of research on effects of fibre intake on man, *Am J Clin Nutr*, 1978, 31, 142.
2. Selvendran R R, The plant cell wall as a source of dietary fibre: Chemistry and structure, *Am J Clin Nutr*, 1984, 39, 320.
3. Southgate D A T, Determination of carbohydrates in foods — I. Available carbohydrates II. Unavailable carbohydrates, *J Sci Fd Agric*, 1969, 20, 326.
4. Van Soest P J, Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fibre and lignin, *J. ass. Off. analyt. Chem., 1963, 46, 829.*
5. Van Soest P J. and Wine R H, *Use of detergents in the analysis of fibrous feeds. IV Determination of plant cell wall constituents, J. ass. off. anal: Chem.*, 1967, 50, 50.
6. Hellendoorn, E W., Noordhoff, M S. and Slagman, J. Enzymatic determination of the indigestible residue of human food, *J Sci Fd Agric.*, 1975, 26, 1461.
7. Kamath V M and Belavady B, Unavailable carbohydrates of commonly consumed Indian foods, *J Sci Fd Agric*, 1980, 31, 194.
8. Prema Kumari M N, Fathima A and Saraswathi G, Dietary fibre content of some food materials, *J Fd Sci Tech.*, 1984, 21, 95.
9. Saraswathi G, Kanchana S. and Shurpalekar K S, Estimation of dietary fibre of some selected Indian foods by different methods, *Ind. J Med Res*, 1983, 77, 833.
10. Candlish J K, Gourley L. and Lee H P, Dietary fiber and starch in some Southeast Asian Fruits, *Fd Comp Analy.* 1987, 1, 81.
11. *Official Methods of Analysis*, Association of Official Analytical Chemists, Virginia. 1984, 249 and 162.
12. Snedecor G W and Cochran W G, *Statistical Methods*, Oxford and I.B.H. Publishing Co., London, 1967, 6th Edn.
13. Horvath P J and Robertson J R, Dietary fiber content of selected foods, In *CRC Hand Book of Dietary Fiber in Human Nutrition* G A Spiller, (Ed.) CRC Press Inc. Florida, 1986, 435.
14. Jenkins D J A, Wolever T M S, Hockaday D T R, Leeds A R, Howarth R, Bacon S, Apling E and Dilawari J, Treatment of diabetics with guar gum: Reduction of urinary glucose loss in diabetics. *Lancet.* 1977, 12, 779.
15. Jenkins D J A, Wolever, T M S, Leeds A R, Gassull M A, Haisman P, Dilawari J, Goff D V, Metz G L, and Alberti K G M M, Dietary fiber, fiber analogues and glucose tolerance: Importance of viscosity, *Br. med J.* 1978, 1, 1392.
16. Jenkins D J A, Dietary fibre and other antinutrients: Metabolic effects and therapeutic implications, In *Nutritional Pharmacology* G A Spiller (Ed.). Alan R. Liss, Inc, New York, 1981, 117.

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CHANGES IN DISULPHIDE CONTENT AT DIFFERENT STAGES OF MANUFACTURE OF STERILIZED MILK FROM BUFFALO MILK

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Disulphides of both cow's and buffalo milks increased due to pre-heating and sterilization. The increase in values as a result of sterilization was about two fold. The disulphide content of buffalo milk was observed to be lower than that of cow's milk.

Thermal processes like pasteurization, pre-heating and sterilization of milk result in "exposure" of sulphydryl compounds especially from the milk serum proteins. The denaturation of milk serum proteins resulted in increased values of sulphydryl plus disulphide[1]. Low molecular weight sulphur compounds including disulphides and sulphydryl groups produce distinct cooked flavour in milk and simultaneously increase the oxidative stability of milk system. Although buffaloes contribute more than 57 per cent of total milk production in our country[2], no information is available on the disulphide content of buffalo milk when subjected to pre-heating and sterilization. Therefore, it was considered pertinent to undertake the present study to obtain this information. The results on disulphide content of buffalo milk were compared with those from cow's milk under similar conditions.

Composite milk samples of cow and buffalo from the Institute's Farm (maintaining Indian cows, cross-bred cows and Murrah buffaloes) were processed in the Experimental Dairy of the Institute. After performing platform tests[3] and clarification, the milk was standardised to 4.5 per cent fat and 8.5 per cent SNF for cow's milk and 6.0 per cent fat and 9.0 per cent SNF buffalo milk. Then the milk samples were pre-heated at 100°C/flash and 115°C/flash separately in a Silkeborg plate heater of 2500 lb/h capacity. Thereafter, the samples were subjected to homogenisation at 175 kg/cm^2 pressure in a single stage homogeniser. Sterilization of milk samples was carried out at 115°C for 10 min and 115°C for 15 min in crown corked glass bottles using a batch sterilizer. Three trials each were carried out for treatment of pre-heating and sterilization and separately for both cow's and buffalo milks.

Disulphide contents in raw, pre-treated and sterilized milks were determined by the method of De Marco *et al.*[4]. This method is based upon the reduction of disulphide compounds with the help of KCN as a reducing agent and estimating as sulphydryls. When disulphide is reduced with KCN[4,5], only one mole of free sulphydryl is produced and not two moles of sulphydryl. Hence, the disulphide concentration was calculated without dividing by 2.

The data reveal that the disulphides in raw buffalo milk are less than of raw cow's milk (Table 1). The values increase due to pre-heating upto 100°C but a slight decrease occurs on increasing pre-heating to 115°C. The original values are increased two-fold as a result of sterilization. The trend of increase is similar for both cow's and buffalo milks. For those samples of cow and buffalo milks which received maximum heat treatment during pre-heating at 115°C and sterilization at 115°C for 15 min, the original values of 0.098 and 0.074 increased to 0.194 and 0.148, respectively for cow's and buffalo milks after sterilization.

Disulphide content in milk reported by some workers[6-13] was higher than that in the present investigation. This is due to methodology, initial quality of milk and addition of no dissociating agent in the present study prior to estimation. They found disulphide to be more resistant to heat than thiols. Total sulphydryls plus disulphides in milk increased upon pasteurisation but decreased on UHT sterilization and the sulphur compound contents were correlated with cooked flavour intensity. In the present investigation, sodium

TABLE 1. DISULPHIDE CONTENT AT VARIOUS STAGES IN THE MANUFACTUE OF STERILIZED MILK

Source of milk	Pre-heating temp (°C)	Disulphide content (μ m/ml) of milk			
		Raw milk	Pre-heated milk	Sterilized milk	
				115°C/10 min	115°C/15 min
Cow	110/flash	0.098 ± 0.006	0.122 ± 0.007	0.175 ± 0.008	0.189 ± 0.008
Buffalo	110/flash	0.074 ± 0.006	0.095 ± 0.007	0.126 ± 0.007	0.141 ± 0.008
Cow	115/flash	0.098 ± 0.006	0.116 ± 0.007	0.178 ± 0.007	0.194 ± 0.008
Buffalo	115/flash	0.074 ± 0.006	0.090 ± 0.006	0.132 ± 0.007	0.148 ± 0.008

Three trials each were conducted for treatments of pre-heating and sterilization.
Values are mean ± S.D.

nitroprusside test[14] for cooked flavour was performed on the milk samples. It was negative for cow's and buffalo raw milk, positive for pre-heated milks and strongly positive for sterilized milks.

References

1. Manning P B, Coulter S T and Jenness R, Estimation of whey protein denaturation in milk products of unknown heat treatments *J Dairy Sci*, 1965, 48, 776.
2. Rajorhia G S, Buffalo milk products, *Indian Frmng*, 1987, 37(6), 52.
3. *Test for Dairy Industry, Rapid Examination of Milk*-Part-I, IS: 1479—1960, Indian Standards Institution, New Delhi.
4. De Marco C, Graziani M T and Mosti R, Spectrophotometric determination of thiols and disulphides with N-ethylmalienimide, *Analyt, Biochem*, 1966, 15, 40.
5. Friedman M, *The Chemistry and Biochemistry of sulphydryl Group in Amino Acids, Peptides and Proteins*, Pregamon Press Inc., New York, 1973, 215.
6. Seitov Z S and Seitkaliev K S, Sulphydryl groups in milk, *Tr. almaatin. zootekh, vet inst*, 1970, 18, 16 *Dairy Sci Abstr.*, 1972, 34, 92.
7. Mrowetz G, Klostermeyer H and Thomasow J, Thiol and disulphide groups in milk products; Estimation and results, *Z. Lebens Forschung*, 1972, 149, 341, *Dairy Sci. Abstr.*, 1973, 35, 146.
8. Beveridge T, Toma S J and Nakai S, Determination of sulphydryl and disulphide groups in some food proteins using Ellman's reagent, *J Fd Sci.*, 1974, 39, 49.
9. Yoshino U Wilson H K and Harried E D, Amperometric titration of sulphydryl and disulphide groups in milk proteins, *J Dairy Sci*, 1962, 45, 1459.
10. Sasago K, Wilson H K, and Herried E D, Determination of sulphydryl and disulphide groups in milk by the p-chloromercuribenzoate-dithizone method, *J Dairy Sci*, 1963, 46, 1348.
11. Patrick P S and Swaisgood H E, Sulphydryl and disulphide groups in skim milk as affected by direct ultra-high temperature heating and subsequent storage, *J Dairy Sci*, 1976, 59 594.
12. Aboshama K and Hansen A P, Effect of ultra-heat temperature steam injection processing on sulphydryl containing amino-acids in milk. *J Dairy Sci*, 1977, 60, 1374.
13. Gorner F, Sedlak J and Heldak J, relationship between taste and sulphur compounds in long-life milk. *Polnohospodarstvo* 1978, 24, 916. *Dairy Sci. Abstr*, 1981, 43, 221.
14. Grunert R R and Phillips P H, A modification of the nitroprusside method of analysis for glutathione, *Arch Biochem*, 1951, 30, 217.

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SYNERESIS AND CHEESE YIELD OF BOVINE MILK WITH DIFFERENT CHYMOSIN COAGULATION PROPERTIES[1]

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The rate of syneresis in chymosin coagulated bovine milk blends with added 2% lactic starter was estimated by continuous curd firmness measurement in the Formagraph instrument for 3 hr at 37°C. Attainment of peak curd firmness values in the blends was followed by a progressive decrease in firmness, with a simultaneous shrinkage (syneresis) of coagula. Milk blend with good coagulation properties syneresed more than poor coagulating milk. Linear correlation coefficients r = —.98 were obtained between decreases in curd firmness and syneresis time for good, and poor coagulating milks respectively. Cheddar cheese was made in 9 L laboratory vats with three blends of pasteurized and coagulation improved milk. Poor coagulating milk blend produced cheese with highest moisture, lowest yield and most bitter flavour compared to other blends.

Recent studies have established that a positive relationship exists between curd firmness at cutting and cheese yield[1,2]. An early report[3] indicated that milk which formed a weak coagulum produces low cheese yield. In these studies, there are contradictions as to the relationship that exists between curd firmness at cutting and rate of syneresis during curd cooking. Some workers[2] observed that high curd firmness at cutting causes high moisture retention but increased cheese yield because of more milk fat entrapment in curd. Others[1], however, insist that moisture content of cheese is not affected by curd firmness. In the recent studies cited, mixed milk samples were used and curd firmness values produced were the average effects of milks with good and poor chymosin coagulation properties[4,5]. It is desirable to study the effects of milks with different chymosin coagulation properties on syneresis and cheese yield. These milks could be blended in various known proportions, and thus would provide adequate models for such investigations.

This work summarizes studies on syneresis and cheese yield of milk blends with good and poor chymosin coagulation properties.

Selection of milk samples: Fifty individual cow's milk samples were obtained from the Utah State University Holstein herd at one sampling. Cows in mid- and late-lactation were selected to represent firm and soft curd milk[4]. Each sample (10 ml) was coagulated as described[5]. Four good coagulating samples (GCM) and four poor coagulating samples (PCM) were selected based on curd firmness and source cows were identified. Ten ml each of 4 GCM samples were pooled, and PCM samples were similarly treated. The pH of each pooled sample was measured at 37°C with a calibrated Beckman model Ω 60 pH meter (Beckman Instruments Inc., Fullerton, CA 92634).

Estimation of syneresis rate: Ten ml aliquots of pooled, raw GCM, and PCM were pipetted into test tubes and 1 per cent each of active lactic starters, *Streptococcus cremoris* (UC 310) and *S. cremoris* (UC 77) were added to each aliquot, and all were tempered at 37°C for 90 min. Tempering also served as incubation period for lactic starters to enter their active growth phase. The pH of each sample was measured after tempering at 37°C, and each sample was then coagulated in a Formagraph instrument with 200 μl of 0.4 rennin units (RU) per ml of chymosin. The coagulating milk samples were left in the Formagraph for a total of 180 min during which continuous curd firmness measurements of the samples were made, and the coagula syneresed. Syneresis was manifested as shrinkage of milk coagula on the Formagraph pendulae. Continuous decrease in curd firmness that occurred in the samples after 30 min of chymosin addition, was taken as an estimate of syneresis.

Optimization of coagulation properties of cheese milk blends: Cheese milk was obtained from each of the 8 identified cows which produced GCM, and PCM. GCM from 4 cows was pooled, and PCM was similarly treated. Prior to cheese making, aliquots of the GCM, and PCM were blended as follows: (a) 100 per cent PCM (b) 50 per cent PCM + 50 per cent GCM (c) 100 per cent GCM, and 0.02 per cent calcium chloride was added to each blend. The pH of each blend was reduced to 6.3 with 1 to 3 drops of 2.1 N lactic acid solution. The blends were tempered at 37°C for 90 min during which shifts in pH were adjusted. Each blend was then optimally coagulated in the Formagraph with 200 μl of 0.2 RU/ml of chymosin as previously established[5].

Estimation of cheese yield: Each milk blend (7-5 l) was pasteurized in a 9 l laboratory cheese vat at 63°C for 30 min and 0.02 per cent calcium chloride added. One per cent each of lactic starters UC 310 and UC 77 was also added. These cultures had been separately incubated overnight in pH-controlled whey base medium with added stimulants (yeast extract and casein hydrolysate). Each vat was tempered/ripened until milk pH was reduced to 6.3. One hundred and fifty ml of diluted (.2 RU/ml) chymosin (Chris

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Hansen, Milwaukee, WI.) was added. Each milk blend was well stirred and left to coagulate at 37°C. Traditional cheddar cheese making procedures were then used. Cheese moisture also was determined[7].

Variation in syneresis rate: Curd firmness increased in the blended GCM and PCM samples until after 29.5 and 30 min of chymosin addition respectively when a gradual but progressive decrease in firmness occurred (Fig 1). Curd shrinkage (syneresis) with exuded whey which partly collected in the interface of the Formagraph pendulae and curd, and partly in the interface of the curd and Formagraph cuvettes occurred simultaneously as curd firmness decreased. A progressive collection of whey in these interfaces was observed until the end of the 3 hr Formagraph run. Expression of whey occurs as colloidal calcium phosphate is lost from rennet treated casein micelles in consequence to lowering of pH[8,9]. Major decreases in curd firmness did not occur in coagulated, no-acidified 10 and 14 per cent non-fat-dry milk (NFDM), and no visible changes in the form of curd shrinkage and whey collection were observed in the Formagraph cuvettes that contained them. It was remarkable to note that the final curd firmness value in GCM (33 mm) after 3 hr was higher than the peak value in PCM (28 mm) which occurred after 29.5 min of chymosin addition. GCM showed more curd shrinkage than PCM as was visibly observed at the end of the Formagraph run. Linear correlation coefficients r = −0.98 and −0.99 were obtained between decreases in curd firmness (syneresis) and syneresis time for GCM and PCM respectively with corresponding average rates of decrease in curd firmness of 0.15 and 0.12 mm/min. The steeper slope of decrease in curd firmness per unit time recorded for GCM compared to PCM indicates that syneresis rate is higher in GCM than PCM.

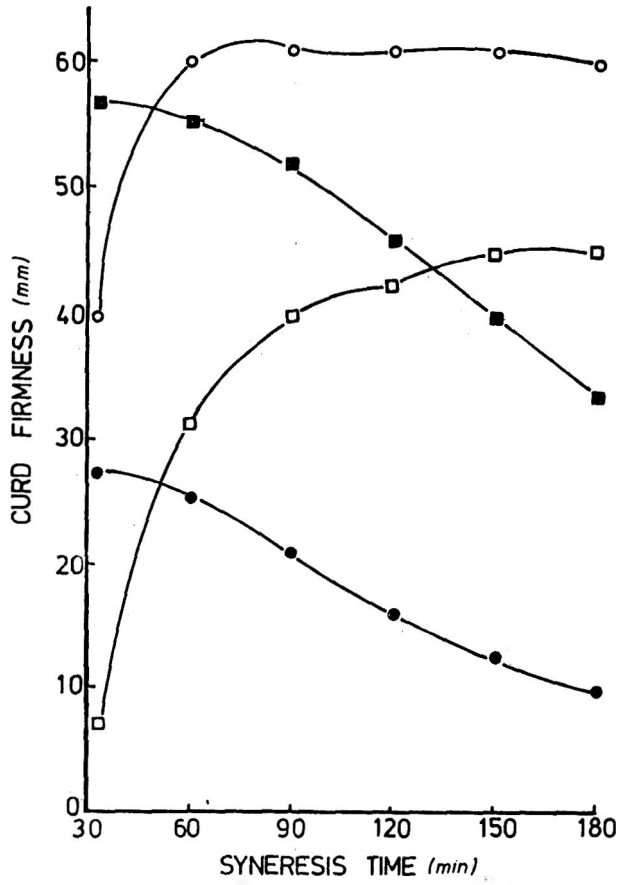

Fig.1. Curd firmness values during syneresis of chymosin coagulating milks (■ blend of good coagulating milk, ● blend of poor coagulating milk, each with added 2% lactic starter; □ 10% and ○ 14% reconstituted non-fat dry milk without starter).

Tuszynski *et al.*[8] similarly evaluated the softening of milk gels with thrombelastograph, and they attributed it to "retraction" or syneresis. Curd softening occurs characteristically at initial pH values of 6.02 to 5.94, and higher rates of softening have been observed at pH 5.2[8]. Milk that was acidified to pH 5.6 before enzyme addition, produced curd which either stabilized in firmness at 30 min or exhibited a decay[9]. If cheese milk is to be acidified, it is suggested that the pH after acidification be above 6.02 because curd produced by milk at or below this pH would synerese poorly. The initial pH values of GCM and PCM before acidification were 6.63 and 6.88, and after acidification with culture were 6.22 and 6.51 respectively before chymosin addition. After 3 hr coagulation, the final pH values were 5.11 and 5.55 for GCM and PCM, corresponding to a total incubation time of 4.5 hr at 37°C.

Improper coagulum formation by PCM has been attributed, primarily, to conversion of ß-casein into cleavage products which include γ -caseins and proteose peptones probably by indigenous milk enzymes[10]. These products were apparently absent in whey and very likely were trapped in curd together with milk fat globules. Improper bond formation in PCM may have caused poor curd formation resulting in reduced syneresis rate. Hill and Merrill[3] attributed high moisture retention in cheese to soft curded milk.

Cheese yield: Cheese yield (dry weight) ranged from 3.39 to 5.32 per cent for the milk blends (Table 1). Moisture was highest, 59.2 per cent in cheese made with 100 per cent PCM and least, 41.4 per cent in 100 per cent GCM cheese. High moisture level in cheese made from 100 per cent PCM agrees with syneresis data in Fig. 1 in which PCM showed a lower rate of curd firmness decrease than GCM. Eventhough PCM was improved to produce curd firmness value of 47 mm, and its curd was ready to cut 30 min after chymosin addition, subsequent syneresis after cutting was poor. Curd firmness values for GCM, and GCM-PCM blends were 54 and 52 mm respectively. In as much as GCM and GCM-PCM produced high curd firmness, higher cheese yield was obtained with the latter. This suggests that yield is not entirely dependent

TABLE 1 CHEESE YIELD ESTIMATES FO MILK BLENDS WITH DIFFERENT CHYMOSIN COAGULATION PROPERTIES.

Parameter	Milk blends		
	PCM[1]	50 % PCM + 50 % GCM	GCM
Curd firmness[2] (mm)	47	52	54
Cheese yield (%) (wet wt)	8.32	9.37	8.94
Cheese yield (%) (dry wt)	3.39	5.32	5.24
Cheese moisture (%)	59.20	43.20	41.40

[1] PCM: poor chymosin-coagulating milk
GCM: good chymosin-coagulating milk

[2] Formagraph reading 30 min after chymosin addition obtained in a preliminary study utilizing the same coagulation conditions (chymosin concentration 0.2 RU/ml, milk pH = 6.3, calcium chloride = .02 per cent, and temperature = 37°C) to insure adequate coagulation in PCM.

on curd firmness. A cheese yield formula with components of casein, fat, moisture and curd firmness may be desirable. Cheese made from 100 per cent PCM was of lowest yield, highest moisture and very bitter flavour as was observed by an informal taste test. PCM has been strongly associated with late lactation milk[4,5], and high proteolytic activities also have been established in late lactation milk[11-14]. High proteolytic activities in PCM[10] have been established. casein cleavage products in late lactation milk and PCM might be partly responsible for rapid deterioration of high moisture cheese made from soft curd milk[3]. Significant quantities of cleavage products are expected in bulk milk supplies containing substantial amounts of PCM. Deterioration of curd firmness of cold stored bovine milk has also been related to PCM[15]. PCM could be shunted for non-cheese uses depending on the extent of its inability to coagulate with chymosin. Further work is needed to compare the organoleptic quality of cheese made with PCM and that of GCM. Subsequent biochemical changes that occur during curing of both cheeses should also be investigated.

References

1. Bynum D H and Olson N F, Influence of curd firmness at cutting on cheddar cheese yield and recovery of milk constituents. *J Dairy Sci*, 1982, 65, 2281.
2. *Cheese Technology, Report of Research 1981-1982.*, Commonwealth Scientific and Industrial Research Organization Publ., Sydney, Australia, 1982, 79.
3. Hill R L and Merrill A C, The manufacture of cheese from soft curd and hard curd milks, *Utah Agric Exp Sta BUll*, NO 236, 1932.
4. Okigbo L M, Richardson G H Brown R J and Ernstrom C A, Variation in coagulation properties of milk from individual cows. *J Dairy Sci*, 1985, 68, 822.
5. Okigbo L M Richardson G H Brown R J and Ernstrom C A, Effects of pH, calcium chloride, and chymosin concentration on coagulation properties of abnormal and normal milk. *J Dairy Sci*, 1985, 68, 2527.
6. McMahon D J and Brown R J, Evaluation of the Formagraph for comparing rennet solutions *J Dairy Sci*, 1982, 65, 1639.
7. Shehata A E Iyer M Olson N F and Richardson T, Effect of type of acid used in direct acidification procedures on moisture, firmness, and calcium levels of cheese. *J Dairy Sci*, 1967, 50,824.
8. Tuszynski W Burnett J and Scott-Blair G W, The effects of variations in pH, of the removal of calcium and of the addition of sulphur-bond inhibitors on the rate of setting of renneted milk. *J Dairy Res*, 1968, 35, 71.
9. Olson N F and Bottazzi V, Rheology of milk gels formed by milk clotting enzymes, *J Food Sci, 1977, 42, 669.*
10. Okigbo L M, Richardson G H Brown R J and Ernstrom C A, *Casein composition of cow's milk of different chymosin coagulation properties. J Dairy Sci*, 1985, 68, 1887.
11. Davies D T and Law A J R, The composition of whole casein for milk of Ayshire cows. *J Dairy Res*, 1977, 44, 447.
12. Donnelly W J and Barry J G, Casein compositional studies, III, Changes in Irish milk for manufacturing and role of milk proteinase. *J Dairy Res*, 1983, 50, 433.
13. Reimerdes E H, New aspects of naturally occurring proteins in bovine milk *J Dairy Sci*, 1983, 66, 1591.
14. Richardson B C, Variation of the concentration of plasmin and plasminogen in bovine milk with lactation. *N Z J Dairy Sci Technol*, 1983, 18, 247.
15. Nsofor L M, Variation in curd firmness of cold stored raw milk from individual cows. *J Fd Sci, Technol*, 1989, (In press).

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EFFECT OF LACTIC ACID AND POTASSIUM SORBATE DIP ON REFRIGERATED SHELF-LIFE OF DRESSED QUAIL

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The effect of dipping of whole quail carcasses in 2% lactic acid or 2% lactic acid plus 2% potassium sorbate solutions on their refrigerated (4±0.5°C) shelf-life was investigated. Acid treatment had greater effect on the initial reduction in pH of skin than meat. Both skin and meat showed pH rise at the time of spoilage. TBA number increased consistently with storage time regardless of treatments. Treated carcasses had about on log cycle less total viable counts than control throughout the storage. Chemical dip treatments appeared to have limited potential in extending the shelf-life of dressed quails upto 10 days in contrast to 8 days for control at 4±0.5°C.

The shelf-life of poultry meat at chill temperature is influenced by the initial microbial load, holding environment and associated chemical changes in meat during storage. Organic acidulants [1-3] and potassium sorbate have been extensively used as a dip or spray for extending the storage life of poultry at low temperatures. Apart from its antimycotic property, potassium sorbate has been reported to exert antibacterial effect [4-7] on dressed poultry. The present study was, therefore, undertaken to assess the effect of lactic acid dip either alone or in combination with potassium sorbate on the keeping quality of quail carcasses under refrigerated (4±0.5°C) storage.

Five-week old quails (*Coturnix coturnix japonica*) were conventionally dressed, chilled in slush-ice for 2 hr and drained for 15 min. Whole carcasses, excluding giblets, were dipped in either 2 per cent lactic acid or 2 per cent lactic acid plus 2 per cent potassium sorbate solutions in the ratio of 1:1 (w/v) for 2 min and drained for 15 min. After draining, both control (dipped in distilled water) and treated carcasses were individually packaged in high density polyethylene (330 G) pouches, sealed and stored at refrigerated (4±0.5°C) temperature.

The pH of ground meat and skin samples diluted five times their weight with distilled water was estimated in duplicate using a Beckman pH meter. Thiobarbituric acid (TBA) values were estimated in triplicate according to Tarladgis *et al* [8]. and total aerobic plate count [9] was done on duplicate samples. A seven-member semi-trained sensory panel of the institute evaluated the meat for colour, flavour, tenderness and overall acceptability on a 7-point Hedonic scale. All these analyses were carried out on alternate days until carcasses developed apparent sign of spoilage.

The initial pH values of leg and breast meat from control carcasses within 3 hr of exsanguination were 6.76 and 6.41, respectively. Lactic acid treatment resulted in reduction of skin pH from 6.84 to 5.16 and that of dark and light meat by 0.2 and 0.6 units, respectively. Both control and treated carcasses showed rise in pH of skin as well as meat during later part of storage possibly due to the formation of alkaline substances by proliferating microflora [10].

As shown in Table 1, thiobarbituric acid (TBA) value increased progressively with storage time regardless of treatments. Leg meat had consistently higher TBA value than breast meat. Pikul *et al* [11]. reported that leg meat because of its higher total fat content exhibited higher TBA value than breast meat eventhough the former contained less proportion proportion of phospholipids and consequently less malonaldehyde than the latter.

Lactic acid either singly or in combination with potassium sorbate brought about one log cycle initial reduction in total viable counts per cm^2 compared to control carcasses

TABLE 1. EFFECT OF CHEMICAL DIP ON TBA, TPC AND OVERALL ACCEPTABILITY SCORES OF QUAIL MEAT.

Storage period (days)	Control		Lactic acid		Lactic acid+Pot. sorbate	
	Leg	Breast	Leg	Breast	Leg	Breast
	TBA (mg malonaldehyde/kg)					
0	0.11	0.08	0.14	0.06	0.08	0.05
2	0.18	0.11	0.16	0.11	0.13	0.09
4	0.48	0.39	0.54	0.35	0.41	0.29
6	.1.52	1.20	1.38	1.18	.1.24	1.03
8	2.16	1.67	1.97	1.59	1.61	1.39
10	-	-	2.32	2.04	2.17	1.82
S.E.+	(0.02-0.09)	(0.03-0.11)	(0.02-0.07)	(0.02-0.10)	(0.01-0.12)	(0.02-0.08)
	Total aerobic plate count (log/cm^2)					
0	2.30		1.30		1.39	
2	2.63		1.80		1.86	
4	3.30		2.01		2.05	
6	3.49		2.43		2.39	
8	5.89		4.95		4.94	
10	7.90		6.67		6.64	
S.E.+	(0.02-0.15)		(0.00-0.23)		(0.01-0.09)	
	Overall acceptability scores*					
0	6.71		6.57		6.71	
2	6.28		6.43		6.43	
4	5.57		6.14		6.00	
6	5.28		5.86		5.71	
8	4.28		5.14		5.28	
10	-		4.43		4.57	
S.E.+	(0.22-0.48)		(0.18-0.43)		(0.22-0.40)	

+ = Range *7 = Like very much; 1 = Dislike very much

(Table 1). The identical rate of bacterial multiplication observed between lactic acid and lactic acid plus potassium sorbate treated groups indicated that addition of 2 per cent sorbate was not effective in controlling spoilage at 4 ± 0.5°C. Control carcasses could not be stored beyond 8 days as they developed slime and off-odour on 10th day by which time the total aerobic plate count reached to log 7.9. In contrast, both lactic and lactic acid plus sorbate treated carcasses attained a mean count of log 6.6 on 10th day and developed off-odour on 12th day. Even then, no slime formation was apparent and off-odour was less pronounced than that found on control carcasses. Patterson *et al* [3]. reported that lactic acid or potassium sorbate dip had limited antibacterial effect at 4°C, On the contrary, several studies[4-7] indicated marked improvement in the storage life of whole poultry carcasses or portions dipped in 5-10 per cent potassium sorbate solutions at 4-5°C.

Sensory evaluation indicated that lactic acid or lactic acid plus potassium sorbate treatments had no adverse effect on colour, flavour, tenderness and overall acceptability (Table 1) of quail meat. A progressive decline in scores of all these sensory attributes, except tenderness, occurred with storage time. Spoilage odour became apparent in control carcasses on 10th day in contrast to treated carcasses on 12th day of storage.

These results show that neither 2 percent lactic acid singly nor in combination with 2 per cent potassium sorbate was found to extend the shelf-life of quail carcasses beyond 10 days as against 8 days for untreated control at 4 ± 0.5°C.

References

1. Mountney G J and O'Malley J, Acids as poultry meat preservatives, *Poult Sci*, 1965, 44, 582.
2. Juven B J, Cox N A, Mercuri A J and Thompson J E, A hot acid treatment for eliminating Salmonella from chicken meat, *J Milk Fd Technol* 1974, 37, 237.
3. Patterson J T, Gillespie C W, and Hough B, Aspects of the microbiology of vacuum-and gas-packaged chicken including pre-treatments with lactic acid and potassium sorbate *Br Poult Sci*, 1984, 25, 457.
4. Robach M C, and Ivey F J, Antimicrobial efficacy of a potassium sorbate dip on freshly processed poultry, *J Fd Prot* , 1978, 41, 284.
5. Cunningham F E, Shelf-life and quality characteristics of poultry parts dipped in potassium sorbate. *J Fd Sci*, 1979, 44, 863.
6. To E C, and Robach M C, Potassium sorbate dip as a method of extending shelf-life and inhibiting the growth of *Salmonella* and *Staphylococcus aureus* n fresh, whole broilers, *Poult Sci*, 1980, 59, 726.
7. Lee S H and Han S K, Effect of potassium sorbate on shelf-life and psychrotrophic flora of fresh poultry, *Korean J Anim Sci*, 1986, 28, 742.
8. Tarladgis B G, Watts B M, Younathan M T, and Dugan J R. L, A distillation method for the quantitative determination of malonaldehyde in rancid foods, *J Am Oil Chem Soc*, 1960, 37, 403.
9. Speck M L, *Compendium of Methods for Microbiological Examination of Foods*, APHA Inc. Washington, D.C.
10. Jay J M, and Shelef L A, Microbial modification in raw and processed meats and poultry at low temperatures, *Fd Technol*, 1978, 32, 186.
11. Pikul J, Leszczynski D E, and Kummerow F A, Influence of fat content and composition on malonaldehyde concentration in chicken meat and skin, *Poult Sci*, 1985, 64, 311.

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EFFECT OF DIETARY FIBRE ON PROTEIN QUALITY AND ENERGY AVAILABILITY

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Protein digestibility and energy availability from casein diets containing 3, 6 and 9 % neutral detergent fibre (NDF) from wheat bran and legume husks were studied by feeding albino rats. Protein digestibility was negatively correlated (r = −9.81) with NDF content of the diets. Protein efficiency ratio was higher (P<0.01) at 6 and 9% of NDF compared to reference and 3 % NDF diets. Per cent energy availability at 9% NDF was lower (P<0.01) than the other diets.

Protein energy malnutrition is a serious problem among children in developing countries. This is especially important among vegetarian sections of the population consuming low energy and high fibre diets[1]. Dietary fibre implies indigestible cellulose, hemicellulose, lignin, mucilage, pectin and gum naturally present in foods. There is sufficient evidence to show that fibre in the diet increases stool weight and decreases transit time thus affecting digestion and absorption of nutrients[2,3]. In the present investigation, an attempt has been made to study the effect of different levels of dietary fibre from wheat bran and legume husks on protein digestibility and energy availability.

Rat diets were formulated with three different levels of dietary fibre. These levels were based on the fibre content of the diets consumed by children in an earlier study[4]. Diets were prepared with casein containing 10 per cent protein and 10 per cent refined groundnut oil. Vitaminised starch and salt mixture were prepared as described by Chapman *et al*[5]. and Hawk and Oser[6] and were added to the diets at 1 and 4 per cent levels, respectively. Dietary fibre source in the test diets was a blend of wheat bran, blackgram and greengram husks in the ratios simulating children's diets (87.4 : 6.3 : 6.3)[4]. The calculated neutral detergent fibre (NDF) content of the experimental diets was kept at 3, 6 and 9 per cent levels. Cellulose was used as a source of dietary fibre in the reference diet at recommended level of 5 per cent[5]. The diets were fed *ad libitum* to 3 weeks old weanling albino rats weighing 33.7 ± 6.9 g for a period of 28 days and protein efficiency ratio (PER) was calculated as described by Evans and Witty[7]. Nitrogen was estimated in food and faeces using macro-kjeldahl method[8]. Energy content of food and faeces was determined using bomb calorimeter. The apparent protein digestibility was calculated as described by Evans and Witty[7]. Energy availability was calculated from differences in food and faecal gross energy and expressed as per cent of dietary energy. The data were statistically analysed using 'analysis of variance' and coefficients of correlation.

The data in Table 1 indicate that the protein intake, weight gain and PER values were significantly higher (P< 0.01) in the test diets containing 6 and 9 per cent NDF compared to reference diet. The values for these parameters for 3 per cent NDF diet were not different from reference diet. The increase in NDF content in test diets resulted in corresponding increase in daily protein intake. Desmoulin *et al*[9]. and Rao and Sundaravalli[10] reported that the rats spontaneously adjusted their food intake to meet the requirements fo major nutrients. Ahrens *et al*[11]. and O' hea *et al*[12]. also reported that food intake increased with an increase in dietary fibre to keep the energy balance. PER values in 6 and 9 per cent NDF containing diets were higher because of higher daily protein in diet due to more food consumption. Fleming and Lee[13] studied the effect of purified natural dietary fibres on rat growth and reported that cellulose and hemicellose containing xylose had a beneficial effect on the PER values. Nomani *et al*[14] have also reported that diets with wheat bran (5 per cent) and neutral detergent fibre (2.1 per cent) showed higher weight gain and PER compared to no fibre diet. Similarly inclusion of 8 per cent ragi (*Ekusine coracana*) husk at 9 per cent casein increased weight gain significantly, but not at 18 per cent casein[15]. Reference casein diet showed PER value of 1.86 which was lower than the standard value and it might be the result of processing conditions of casein. Similar low value of PER for casein (1.85 ± 0.23) has also been reported by Chopra and Hira[16].

Protein digestibility and energy availability of diets decreased with increase in dietary fibre (Table 1) and corresponding values for coefficients of correlations being −0.81 and −0.84, respectively. Increase in dietary fibre from 3 to 6 and 6 to 9 per cent decreased the protein digestibility significantly (P<0.01) whereas decrease (P<0.01) in energy availability was observed only when fibre content was increased from 6 to 9 per cent in the diet. The apparent protein digestibility of 3 per cent NDF diet from dietary sources was lower than that of the reference diet containing 5 per cent NDF as cellulose. Energy absorption from reference diet was not significantly different from 3 per cent NDF diet. Cellulose and cell wall fibre fractions are known to decrease apparent protein digestibility due to higher faecal excretion of nitrogen coming from cell wall bound nitrogen of the bran and lignified nitrogen[13,14]. In spite of low digestibility, dietary fibre inclusion results in improved utilisation of nitrogen at marginal levels of protein and

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TABLE 1. EFFECT OF DIETARY FIBRE ON PROTEIN DIGESTIBILITY AND ENERGY AVAILABILITY[1]

NDF in diet (%)	Protein intake (g)	Gain in wt (g)	PER	Protein digestibility (%)	Energy availability (%)
5.00[+]	13.7 ± 2.8	25.5 ± 4.7	1.86 ± 0.2	87.5 ± 1.4	92.1 ± 0.5
3.00	17.1 ± 3.3	35.8 ± 5.2	2.09 ± 0.5	85.4 ± 1.8	92.1 ± 1.0
6.00	20.5 ± 3.2	48.3 ± 3.9	2.36 ± 0.3	83.0 ± 1.1	91.5 ± 0.6
9.00	22.3 ± 3.8	52.8 ± 4.4	2.36 ± 0.2	80.6 ± 1.4	87.4 ± 0.7
Variance F-ratio	10.31**	13.9**	6.13**	20.47**	82.50**
C.D. at 5%	3.22	9.75	0.29	1.54	0.84
C.D. at 1%	4.38	13.26	0.40	2.10	1.14

1 Mean ± SD values are average of 8 rats in each group.
+ Fibre in the control diet was cellulose.
** Significant at 1% level.
NDF: Neutral detergent fibre

energy[14] a nd it has also been indicated by higher PER in higher fibre diets in the present study.

According to FAO/WHO report[17] the coefficient of digestibility for whole wheat and legume proteins ranged from 79-90 per cent and that for animal proteins was above 90 per cent. The digestibility coefficient of proteins in the present study was within the wheat-legume proteins but lower than that of animal proteins. Though the source of protein was casein, additional fibre from cereals and legumes might have lowered the digestibility coefficient. The energy availability of the diets decreased by 4 per cent with an increase in NDF content from 6 to 9 per cent and the value of 87.4 per cent energy availability at 9 per cent NDF was lower than the FAO/WHO[17] value of 90-97 per cent energy utilization from cereals and legumes.

Thus, it could be concluded that 6 per cent NDF diet has significantly higher PER value. The energy and protein availability were also within the limits prescribed by FAO/WHO. The diet containing 9 per cent NDF has significantly low energy availability with no improvement in PER value. Thus, to utilise maximum energy, NDF content should not be more than 6 per cent in the children's diets.

References

1. Kelsay J L, A review of research on effect of fibre intake on man. *Am J Clin Nutr*, 1978, 31, 142.
2. Southgate D A T, Fibre and other unavailable carbohydrates and their effects on energy value of the diet. *Proc. Nutr Soc*, 1973, 32, 131.
3. Reinhold J G, Faraji B, Abadi P, and Beigi I F, Decreased absorption of calcium, magnesium, zinc and phosphorus by human subjects due to increased fibre and phosphorus consumption as wheat bread. *J Nutr*, 1976, 106, 493.
4. Jain R, *Effect of Dietary Fibre on Energy and Protein Utilisation in Children*. M.Sc. Thesis, 1984, Punjab Agricultural University, Ludhiana.
5. Chapman D G, Castillo P, and Campbell J A, Evaluation of protein in foods. A method for determination of protein efficiency ratio. *Can J Biochem Physiol*, 1959, 37, 679.
6. Hawk P B, and Oser B L, *Hawk's Physiological Chemistry*, McGraw Hill, N Y 1965, 4th Edn. 1373.
7. Evans F, and Witty R, An assessment of methods used to determine protein quality. *Wld Rev Nutr Dietet*, 1978, 32, I.
8. *Official Methods of Analysis*, Association of Official Analytical Chemists, Washington D C, 1980, 13th Edn.
9. Desmoulin B, Delio P, Lecourtier M, and Peiniau P, Comparison of methods of rationing feed according to the type of source of energy in the diet. *Ann Biol Anim Biochim Biophys*, 1970, 10, 101.
10. Rao M N, and Sundaravalli O E, Cellulose effect on protein utilisation - Studies with weanling rat. *J Am Dietet Assoc*, 1970, 57, 517.
11. Ahrens R A, Brown E G, Garlson D E, Porter L B, and Slowen D D, Tissue composition changes in mature rats fed a cellulose bulking agent. *J Home Eco Rec*, 1972, 1, 13.
12. O'hea, E K, Aldis A E, Skidmore G B, Smith B H, Stevenson S, Strongitharam D and Stuart G C F, Effect of dietary dilution on food intake, weight gain, plasma glucose and insulin and adipose tissue insulin sensitivity in rats. *Comp Biochem Physiol*, 1974, 48 A, 21.
13. Fleming S E, and Lee B, Growth performance and intestinal transit time of rats fed purified and natural dietary fibre. *J Nutr*, 1983, 113, 592.
14. Nemani M Z A, Fashandi E F, Davis G K and Bradac C K, Influence of dietary fibre on the growth and protein metabolism of the rat. *J Fd Sci*, 1979, 44, 745.
15. Kanchana S and Shurpalekar K S, Influence of ragi (*Eleusine coracana*) husk on the growth and body composition of albino rats. *Nutr Rep Intern*, 1982, 25, 205.
16. Chopra N and Hira C K, Effect of roasting on protein quality of cereals. *J Fd Sci Technol*, 1986, 23, 233.
17. FAO/WHO *Energy and Protein Requirements*. WHO Technical Report Series No.522, FAO Nutrition. Meetings Report Series No.22, WHO, Geneva, 1973.

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A NOTE ON AFLATOXIN BIOGENESIS IN THE PRESENCE OF BENOMYL AND CARBENDAZIM

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Aflatoxin biogenesis by *A. parasiticus* in the presence of benomyl and carbendazim was studied *in vitro*. Sub-inhibitory concentration of the fungicides stimulated biosynthesis of all the four aflatoxins (B_1, B_2, G_1 and G_2) in culture. At higher concentrations of the fungicides, the fungus lost the ability to form mycelial mat. Instead, abnormal growth in the form of giant cells and a poorly differentiated mycelium was observed in such cultures, which failed to produce aflatoxin.

Pre-harvest crop infection by aflatoxin producing fungi is being increasingly reported[1]. Production of mycotoxins in standing crop and stored grains could be prevented by eliminating mycoflora. A number of chemical fungicides have been developed for use in agriculture. Systemic fungicides namely benomyl and carbendazim are widely used[2,3]. However, the efficacy of these fungicides on mycotoxinogenesis is not clear. The present report relates to the growth and aflatoxin producing potential of *Aspergillus parasiticus* spores exposed to these fungicides.

Aflatoxin producing *Aspergillus parasiticus* NRRL 3145 was used in these studies. The recultivation of the stock culture of the fungus was carried out by streaking the slant material on potato-dextrose agar (PDA) slants. In order to maintain the cultures, regular transfers to fresh slants were carried out at monthly intervals. The spore suspension of the above culture was prepared by transferring the slant material to Roux bottles containing PDA (pH 5.6, 200 ml), which were subsequently incubated for 10 days at ambient temperature (28—30°C). The harvesting of spores from Roux bottles and preparation of spores suspension was carried out as described earlier[4]. The synthetic growth medium for *A. parasiticus* contained (per litre) 200 g glucose, 6.0 g $(NH_4)_2SO_4$; 5.0 g KH_2PO_4, 0.5 g $MgSO_4$, 2.0 g glycine, 2.0 g glutamic acid, 10.0 mg $FeSO_4.7H_2O$, 5.0 mg $ZnSO_4.7H_2O$ and 1.0 mg $MnSO_4.7H_2O$. The complete medium was prepared by mixing sterilized glucose solution with sterilized glucose-free salt solution. The pH of the medium was 6.5. For inoculation, one ml aliquots of the spore suspension were added to flasks containing 50 ml medium in 100 ml conical flasks.

Commercial fungicides, benomyl (methyl 1-(butyl-carbamoyll)-2-benzimidazole ·carbamate) (DUPONT) and carbendazim or Bavistin (BASF) 2-(methoxy-carbamoyl)-benzimidazole having 50 per cent active ingredient in wettable powder were used in these studies. A 1 per cent solution of these fungicides was prepared in ethanol. The solution was filtered through Whatman 542 filter paper and the filtrate was then sterilized by passing through a millipore filter. Measured aliquots, 0.1, 0.2 and 0.3 ml of the sterilized filtrates containing 0.8, 1.6 and 2.4 μ g/ml of fungicide were used in the *in vitro* studies for studying the growth and aflatoxin biogenesis in culture. The concentrations of fungicides were calculated on the basis of solubility of benzimidazole, which is 0.04 g/100g of ethanol at 20°C (BASF India, Tech. Inf. Rep.).

The contents of the culture flasks were filtered through Whatman 541 paper. The resultant filtrate was treated with ferric gel (100 ml water, 10 ml of 10 per cent ferric chloride solution and 15 ml of 4.8 per cent sodium hydroxide solution) in order to remove interfering pigments. The mixture was filtered through Whatman No. 1 paper. The filtrate was extracted with chloroform (2 × the volume of filtrate). The chloroform extracts were evaporated on a water bath (80°C). The residue so obtained was preserved for the estimation of aflatoxin. Total aflatoxin was estimated by using micro-column method as described earlier[4]. For this, a portion of the above residue was suitably diluted with chloroform to bring the reading within 50 ppb range of the fluorotoxin meter. One ml aliquot of the sample was used for loading micro-columns. The micro-column was washed thrice with one ml chloroform. The micro-column with florisil trapped aflatoxin was inserted in the fluorotoxin meter (Velasco flurotoxin meter, Neotec Instruments Inc., MD) and reading was taken. Another reading was taken by turning the micro-column by 180°. The final aflatoxin reading, was the average of the two. From this reading total toxin in the medium was calculated. Quantitation of individual aflatoxin components was carried out by high performance liquid chromatography (HPLC). HPLC was carried out in a liquid chromatograph, Model/GPC-204 (Waters Associates, Millford, MA), with 6000°A pump; U6K septumless injector; Model 440 absorbance detector (365 nm filter); column; waters μ porosil/ bondapak (Silica gel 10 m particle size, 4 mm id, 30 cm long). The solvent was prepared from a water saturated dichloromethane-cyclo- hexane-acetonitrile (25 : 7.5 : 1.0) stabilized with 2 per cent 2-propanol[5]. The sample residue obtained above was dissolved in 0.1 ml of the HPLC solvent and 10 or 2 l aliquots were used for injection into the liquid chromatograph.

Table 1 shows the effect of two systemic fungicides of benzimidazole carbamate group on growth and aflatoxin biogenesis in the cultures of *Aspergillus parasiticus*. High concentrations of carbamates inhibited mycelial growth and also the toxin biogenesis. However, sub-inhibitory concentrations stimulated the toxin production.

HPLC analysis of aflatoxins showed that low concentrations (0.8 μ g/ml)of the fungicide stimulated biogenesis of all the

TABLE 1. EFFECT OF SYSTEMIC FUNGICIDES OF CARBAMATE GROUP ON GROWTH AND AFLATOXIN PRODUCTION IN CULTURES OF *A. PARASITICUS* NRRL 3145

Fungicide	Fungicide concn (μg/ml)	Growth (g)	Aflatoxin (μg/50 ml)
Control	0	1.25	900
Carbendazim (Bavistin)	0.8	1.45	1350
Carbendazim	1.6	1.17	490
Carbendazim	2.4	0	0
Benomyl	0.8	1.35	1075
Benomyl	1.6	0.61	25
Benomyl	2.4	0	0

Growth expressed as mycelial dry wt, inoculum *ca.* 10^6 spores, 50 ml glucose salt medium, period of incubation 14 days at ambient temp. Aflatoxin measurement by micro-column method. Readings average of three replicates.

TABLE 2. EFFECT OF BAVISTIN ON SYNTHESIS OF DIFFERENT FORMS OF AFLATOXIN BY *A. PARASITICUS* NRRL 3145

Carbendazim concn (μg/ml)	Aflatoxin (μg/100 ml*)				
	B_1	B_2	G_1	G_2	Total
0	840	530	102	290	1762
0.8	1400	770	204	300	2674
1.6	850	150	51	16	967

Estimation of aflatoxin by HPLC, conditions described in text, culture conditions as in Table 1. *For increasing the yield of individual toxin, inoculations were done in 100 ml medium. Readings are the averages of three replicates.

four forms of the toxin (Table 2). The increase was more prominent for B_1 and G_1 toxins, which was about 2 fold over the controls. Inhibition of both growth and toxin biogenesis was observed at higher concentration (2.4 μ g/ml). The growth of the fungus was not apparent in the flasks containing higher amounts of the fungicide. However, during a prolonged incubation upto 30-60 days of these flasks showed turbidity.

Microscopic examination of the medium revealed giant cells and stunted hyphal development. No aflatoxin was detected in the abnormally differentiated cultures. Use of sub-inhibitory concentrations of fungicides could thus be hazardous in view of the stimulation of the toxin biosynthesis. Certain anti-fungal compounds have been observed to stimulate toxin biosynthesis at sub-inhibitory concentrations as reported in our earlier studies with fungicidal compounds from onion and spices[6-9]. The possible mechanism for stimulation has been discussed earlier[4].

Apparently, inhibitory concentrations of fungicides could allow stunted microscopic growth of the fungus, which did not support toxin biosynthesis. Mode of action of anti-fungal compounds varies with the nature of fungitoxic principle. Benomyl and carbendazim are known to interfere with the biosynthesis of DNA[2]. Lack of aflatoxin biosynthetic ability in undifferentiated cells observed above confirms the need for complete differentiation of the mycelium for high yields of the toxin in cultures as pointed out earlier[4].

References

1. Lillehoj E B, Kwolek W F, Zuber M S, Horner B S, Widstrom N W, Guthrie W D, Turner M, Sauer D B, Findley W R, Manwiller A and Josephson L M, Aflatoxin contamination caused by natural fungal infection of pre-harvest corn. *Pl Soil,*, 1980, 54, 469.
2. Siegel M R and Sisler H D, *Antifungal Compounds*, II Marcel Dekker Inc. New York, 1977. Vol. I and II.
3. Deacon J W, *Introduction to Modern Mycology*, John Wiley & Sons, New York 1980.
4. Sharma A, Behere A G; Padwal-Desai S R and Nadkarni G B, Influence of inoculum size of *Aspergillus parasiticus* spores on aflatoxin production, *Appl. environ. Microbiol.* 1980, 40, 989.
5. Pons W A and Franz A O Jr, High pressure liquid chromatographic determination in peanut products, *J ass off analyt Chem*, 1978, 61, 793.
6. Sharma A, Tewari G M; Padwal-Desai S R and Bandyopadhyay C, Factors affecting antifungal activity of onion extractions against producing fungi, *J Fd Sci*, 1981, 46, 741.
7. Sharma A, Ghanekar A S, Padwal-Desai S R and Nadkarni G B, Microbiological status and antifungal properties of irradiated spices. *J agric Fd Chem*, 1984, 32, 1061.
8. Garies M, Bauer J W, Montgelas A V and Gedek B, Stimulation of aflatoxin B_1 and B_2 toxin production by sorbic acid, *Appl Environ Microbiol*, 1984, 47, 416.
9. Stewart R G, Wyatt R D and Ashmore M D, The effect of various antifungal agents on aflatoxin production and growth characteristics of *Aspergillus parasiticus* and *Aspergillus flavus* in liquid medium, *Poult Sci*, 1977, 56, 1630.

BOOK REVIEWS

Food Structure — Its Creation & Evaluation: by J.M.V. Blanshard & J.R. Mitchell, Butterworth Scientific Ltd., Westbury House, Bury Street, Guildford GU2 5 BH; 1988, pp. 504; Price: £75.

This book is a record of lectures given by a distinguished international group of speakers from different Universities and Food Research organisations at a symposium of Easter Schools in Agricultural Sciences, University of Nottingham, U.K.

There is a mixed bag of 26 topics falling into two categories:

a) Structure of foods and their creation and
b) Evaluation of structure

In the first category are discussed the structural properties and stability of mixed and filled gels, biopolymers, emulsions, foams, intermediate moisture foods, etc. A good number of chapters pertain to the creation of fibrous structures of proteins, formation of structured sugar and fat systems of reformed meat products as well as Surimi based foods and discussions about cereal product structure and extrusions studies. The evaluation methods covered in the rest of the chapters are equally varied and highly interesting. On the top of the list and lst chapter is the relevance of food structure — a dental — clinical perspective. This is a new approach and presents an excellent explanation of the importance of masticatory phenomenon, its loss or reduction in the elderly and the geriatric population and how the food structures and textures could be measured in relation to the chewability of foods. Considering the scant bibliography at the end of the chapter, this aspect of food evaluation has not received as much attention from food scientists as some others. The oral perception of texture is yet another view-point presented by a dentist and an anatomist describing the mandibular action and food breakdown in the mouth and swallowing.

Very authoritative treatments have been given to food structure evaluation by light and electron microscopy, measurements of large and small deformations, evaluation of crispness, the interpretation of 'Collapse phenomenon' of low moisture foods and the examination of the sensory — rheological interface. A chapter entitled 'Beyond the texture profile' contains what one would like to know about sensory evaluation of textures and the possible statistical treatments that can be given to the data.

Printed on high quality paper and wonderfully illustrated with excellent photographs and diagrams, this is a highly readable book answering a million questions a food scientist is likely to ask on the development of structured foods.

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Genetic Engineering Fundamentals : An Introduction to Principles & Applications: by Karl Kammermeyar and Virginia L. Clark; Marcel Dekker Inc, 270 Madison Avenue, New York, N.Y. 10016, 1988; pp: 304; Price: US$ 85 (US & Canada), $102 (All other countries).

This book is aimed at the "non experts", chemical technologists and chemical engineers in particular. The contents are organized into 14 short chapters. The first 5 chapters essentially deal with the theoretical background to the subject of genetic engineering. This includes the components of nucleic acids, concepts of the cell, structure of the gene, protein synthesis and enzymes, with special reference to those used in recombinant DNA technology. Chapters 6 to 9 deal with the principles of the basic procedures used in gene cloning and characterization of the clones using standard protocols. Specific treatment is also given to transformation in plant and animal cells. Aspects of Commercialization, Technology and Design, and Precautions and Regulations are covered in chapters 10, 11 and 13 respectively. A short write-up on ethical/environmental aspects could have been included in this book, I have often found that a non-expert is considerably exercised over this issue.

This book fulfils the objective set and the target group for which it is meant. It gives enough details in terms of principles and the actual methodologies involved. It is not, however, a laboratory manual since actual protocols for carrying out the experiments are not given. Anyone with a Bachelor's degree in Chemistry/Biology should be able to read and be benefitted by this book. The level also seems ideal to perform as a text book for the genetic engineering course offered to M.Sc (Biotechnology) students in India. It will also be useful for teachers at the Bachelor's level. The book is compact and a paper-back edition may be within the reach of purchase by individuals in India.

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Legumes : Chemistry, Technology, and Human Nutrition : Ed by Ruth, H. Matthews, Marcel Dekkar Inc., 270, Madison Ave. New York N Y, 10016, 1989: pp. 408; Price: $99.75 (US & Cananda) $119.50 (all other countries)

Grain legumes are increasingly becoming important as sources of dietary nutrients for many millions of people, particularly in the developing countries. Many books on the

production aspects of grain legumes have been published in the past. But there are a limited number of books on nutritional and food technological aspects of grain legumes. The publication of the above mentioned book is very timely and fills several gaps in understanding the areas of common interest ranging from the chemistry to food uses of grain legumes.

This book contains extremely useful information on various aspects of production and utilization of grain legumes. It provides an in-depth understanding and comprehensive views on the chemistry and technology of production, processing properties of various legumes and legume products. Comparisons of nutritional quality, storage stability and food processing properties of various legumes have been illustrated in this book. Antinutritional factors of several grain legumes have been covered and these indeed provide useful information. More importantly this book deals with a number of grain legumes which are commonly consumed as sources of dietary proteins by the people in tropical and semi-arid tropical regions of the world.

Ten chapters have been presented and discussed in this book. Each chapter has been written by well known scientists. Not only these chapters report the results of past studies but contain valuable interpretation of data including areas of future research work. Notable chapters are those which covers the topics on nutrient composition, bioavailability of nutrients and antinutritional factors. Interestingly, nutrient composition data have been discussed for such foods as cooked and canned legumes, sprouted legumes and other legume products including tofu, tempeh, miso, and natto. However, the chapters on culture, genetics, and harvesting and storage of legumes are equally important and have been presented in a comprehensive way. A detailed account of the chemistry and technology of refined oils, protein isolates and protein concentration have been provided in some chapters of this book and researchers of food processing industry will certainly be benefitted by reading this book. Although the book is intended to cover areas of varied interest, researchers and students of nutrition, food technology and food chemistry would find it a very useful for their work.

In conclusion, I would like to emphasize that this book is a valuable source of information and it may also remain as a very useful reference book for many years in the future.

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ICRISAT, PATANCHERU POST
A.P. 502 324

Food Preservation by Moisture Control : Ed. by C. C. Seow, Elsevier Applied Science, New York, 1988, pp. 277. Price: £36.

This book is a collection of selected papers presented at the International Symposium on Preservation of Foods in Tropical Regions by Control of Internal Aqueous Environment held in Penang, Malaysia during September 1987.

A large number of intermediate moisture (IM) foods based on fish, meat, fruit and starch are consumed in the tropics. The technology associated with their production is primarily related to the control of internal aqueous environment by way of additives such as sugar, salt, acid and antimycotics. In comparison to dry foods, these foods have distinct flavour and mouthfeel characteristics. Some of the typical IM foods are : Dendeng, a meat based product in Indonesia; Sambal daging, Bak Kua and Lup cheang, the meat or fish based product in Malaysia; Dodol, Lempok and Wajik, the starch based product in Malaysia and fruit cake in India. The combined effect of moisture, temperature, acidity, type of additives on the growth or arrest of microflora, nutritional, mouthfeel, oxidative and organoleptic properties of these foods is largely unknown. The survival and growth of the microflora associated in these foods are not only dependent on the water activity (a_w) but also the type of additives, pH and temperature. Sometimes, the quantity of the additives and the degree of partial drying necessary for the preservation of these foods are so large that the desired physical and nutritional characteristics are reduced to a great extent. In that case it becomes necessary to use a superficial edible layer to protect these foods.

The book is an attempt to compile papers on the above mentioned aspects of the IM food technology. It contains 14 papers, four of which are on fundamental condition of water present in food, growth of bacteria and fungi under reduced $a_w$environment as affected by pH and temperature and the effect of a_w on lactose crystallization and lysine loss in skim milk powder.

Five papers are on IM foods based on animal origin. Processing and storage stability of several traditional foods of Malaysia, Indonesia, West Africa and Australia are discussed in these papers.

Five of the papers are on IM foods based on plant origin. About 20 to 30 per cent of the tropical fruits are lost due to lack of adequate storage and processing. IM food technology plays a dominant role in preserving these fruits. One of the papers deals with this aspect. A paper discusses the effect of superficial edible layers in controlling the diffusion of sorbic acid and α-tocopherol. The effect of sulfite on reducing the drying rate of fruit leathers has been discussed in one of the papers. A paper is devoted to the textural changes of some of the Malaysian starch-based IM foods. The storage stability of vegetable oils is dependent on the moisture present in the oil, as the hydrolytic and oxidative rancidities are controlled by the moisture. One of the papers discusses the stability of palm oil.

The value of the book lies especially on the papers written on IM moisture food of the tropics. They add to our knowledge and would certainly be useful.

H. DAS
I.I.T., KHARAGPUR

Adulteration of Fruit Juice Beverages: Ed by S. Negy, J.A. Attaway and M.E. Rhodes, (Food Science and Technology Series) Vol.30, Marcel Dekker Inc., New York and Basel, 1988. pp. 563; Price: Not mentioned

The book "Adulteration of Fruit Juice Beverages" is 30th in the Food Science and Technology Series by the publishers. It aims to explore the many international problems associated with combating fruit juice adulteration and to focus on the chemical, instrumental, statistical and computer assisted pattern recognition methods currently available to deter this practice.

The subject matter is divided into six logical progressive sections each addressing a specific area of this complex subject. There are 23 chapters in all authored by 37 leading experts in which the authenticity of as many as 24 fruit juices, concentrates, diluted beverages and wines has been evaluated.

The introductory first part deals in brief with the history of citrus juice adulteration and discusses the Florida Department of Citrus experience in the USA especially with regard to results of their analyses used as indicators of trouble points by the U.S. Food and Drug Administration for punitive action against adulterators.

In part 2 on chemical markers, there are four chapters devoted to use of free amino acid spectrum and formol number, flavanone glycoside distribution patterns and concentration profiles, aroma and flavour components and trace metal patterns as possible markers for the evaluation, differentiation and detection of adulteration and ascertaining authenticity of citrus and other juices.

Part 3 on physical/chemical methods consists of five chapters of which two deal with application of isotope (carbon or oxygen) ratio analysis to problems of fruit juice adulteration while the remaining three deal with methodologies based on major organic acids, sugars and anthocyanins in cranberry juice and apple juice and those based on visible and u.v. absorption and fluorescence spectral characteristics in selected juices and preserves.

Part 4 on statistical, computer and simulation procedures consists of four chapters of which one dealing with statistical procedures describes two new tests for adulteration — the sequential test useful to quality control inspectors and the directional test for technologists, while another discusses the use of Francine statistical method. The remaining two chapters discuss application of pattern recognition methods and simulation modelling in detection of orange juice adulteration.

In part 5 dealing with commodities, detection of adulteration in specific commodities like fruit wines and selected juices and concentrates are discussed in four chapters.

The final section on international evaluation of fruit juice adulteration relates to the many philosophies and regulatory aspects of both juice exporting and importing countries. With a view to discouraging sales of adulterated fruit beverage products, many countries have developed extensive compositional tables to define normal limits of biochemical components within specific fruit juice beverages. Data on several products from five countries are presented in the five chapters pertaining to Germany, Australia, the Netherlands, Spain and Israel respectively wherein the criteria established in these countries for authenticating fruit juices are individually examined.

With numerous illustrations and over 650 references, the book provides an up-to-date, in-depth look at the new concerns and world-wide regulatory developments in this constantly changing area. With the phenomenal increase in the production, marketing and consumption of natural fruit drinks in India observed in recent times, one could expect prevention of adulteration in fruit juices to pose a challenging task for the regulatory personnel in this country in the very near future. In this context, this book should serve as a valuable guide to fruit beverage scientists, manufacturers and exporters, food technologists, nutritionists, Central and State regulatory officials and to students in food science and biochemistry. It will, therefore, be a useful and informative addition to any library devoted to food science and technology.

K.S. JAYARAMAN
D.F.R.L, MYSORE

Food Borne Bacterial Pathogens: by Michael P. Doyle (ed). Marcel Dekker Inc. NY, Basel, 1989; pp. 796; price US$150 (US & Canada); $180 (all other countries)

This is one of the most recent monographs published by Marcel Dekker Inc., in the Food Science & Technology series. Food microbiologists will welcome this reference text book as it contains under one cover much of what one would wish to know about important bacterial pathogens in relation to human diseases. The monograph has sixteen chapters and each one is exclusively devoted to describing the characteristics of one pathogen, the disease caused by it and its epidemiology, its isolation, identification, pathogenicity, virulence and control measures. The presentation is an interesting medley of gram negative and gram positive bacteria arranged by name in alphabetical order. The classical names such as *Clostridium botulinum, Salmonella* and *Vibrio cholerae* vie for importance with recently recognised

pathogens such as *Aeromonas hydrophila, Campylobacter jejuni, Listeria monocytogenes, Vibrio vulnificus, Yersinia enterocolitica,* etc. Pathogens which are likely to be encountered in future years such as some members of *Enterobacteriaceae, Streptococeae Bacillaceae, Corynebacteriaceae,* nontubercularmycobacteria; *Leptospira* and which may be responsible for human and zoonotic diseases and which may probably spread through foods are also mentioned.

This comprehensive treatise summarises the current state of the art in research on these food borne pathogens. It traces the chronological emergence of several bacteria as established food associated pathogens. The scope and coverage in the book are wide and encompass facts and findings which are not only by now well known but also those which are little known. About *Clostridium perfringens,* it says, for example, that it is the most extensively studied anaerobic bacterium pathogenic to man, is more closely associated with gas gangrene in the last 90 years than with food poisoning which fact came to be known only in the 1940's and was confirmed in the 1960's. On the other hand, the book reports that there has been no fully confirmed outbreak of food borne illness attributed to *Aeromonas hydrophila.* It goes on to reveal that *Campylobacter jejuni* is rated to be the most common bacterial cause of gastro intestinal infection in humans; exceeding rates of illness caused by both *Salmonella* and *Shigella* and yet the two former mentioned organisms have just emerged in the 1980's as recognised and confirmed pathogens. *Listeria monocytogenes,* a gram positive rod has become "judged by its economic and public health impact, one of the most important food borne organisms of the current decade surviving in milk after pasteurisation, growing in a wide pH range and even capable of tolerating upto 16% sodium chloride". Between 1969 and 1979, several bacteria viz. *Vibrio parahaemolyticus, E. coli, Shigella* were confirmed as being food borne pathogens. Recognised as possible pathogens but still not fully confirmed even now are some such as *Acetobacter, Alcaligenes, Citrobacter, Klebsiella, Proteus, Edwardsiella* plus members of several families mentioned earlier.

At this rate, there appears to be not a single bacterium left that might be considered harmless if found associated with any food. Food microbiologists and other food scientists cannot be complacent any more when there are these images of a food harbouring an unending array of hazardous bacterial contaminants which they generally tend to ignore or consider them of little significance. The possibility of potential pathogens lurking in all types of foods and for which special detection techniques and methods are yet to be devised is too near and too frightening.

This monograph by its very contents shows that there is need for stricter vigilance in food processing and food related activities for ensuring microbiological safety in catering and food processing establishments in future years.

The literature coverage on each of the bacterial genera described is thorough and there are over 5000 references in this monograph. The scope and contents of the book are also enlarged and enriched by the wealth of experience and insight brought into the various articles by the individual authors, who are experts in their own areas of work. The organisation of information is very methodical and altogether it makes for a wonderful reading.

VIJAYA RAO
D.F.R.L. MYSORE

Nutrient Interactions (IFT Basic Symposium Series): Edited by C.E. Bodwell and John. W. Erdman, Marcel Dekker Inc., 270, Madison Avenue, New 1988; pp. 389; Price: Bound illustrated US$ 59.75 (US & Cananda) $70.50 (All other countries)

Proceedings of the 11th Annual Basic Symposium sponsored by the Institute of Food Technologists and the International Union of Food Science and Technology held in Las Vegas, Nevada on June 15-16, 1987 are presented in this book in 15 chapters.

Each chapter contains discussions on nutrient interactions that are important in human nutrition; highlighting the recent findings in the respective areas.

Interactions between protein and energy and the role of the latter in other nutrient interactions have been discussed in chapter 1. The implications of these reactions on the currently accepted estimates of requirements and efficiency of nutrient utilization are also considered.

Chapter 2, deals with major metabolic fate of amino acids, amino acid flux, protein turnover and metabolic interactions between amino acids with potential nutritional implications.

Current knowledge about physico-chemical reactions of minerals as determinants of bioavailability in foods, effect of processing and physiological interaction of minerals is presented in chapters 3 and 4.

Chapter 5 deals with the interactions involving protein and iron.

The next 3 chapters present detailed discussions on Vitamin-mineral interactions, factors that affect these interactions and the possible effects.

Information on the interactions between utilizable dietary carbohydrates and minerals and fibre and minerals is provided in chapters 9 and 10. The authors have brought out the influences of the interactions on bioavailability of minerals and considerations of the recommended dietary intakes.

Interacting effects of carbohydrates and lipids on metabolism have been reviewed in chapter 11. Recent findings on the effects of omega 3 — fatty acids have also been included.

Chapter 12 discusses in detail the mode of interaction and net effects of Vitamin — Vitamin interaction and toxicological aspects of aluminium, cadmium and lead.

Type of food additive — nutrient interactions and their effects and regulatory concerns have been presented in Chapter 14.

KANTHA SHURPALEKAR
C.F.T.R.I., MYSORE

AFST(I) News

Madras Chapter

The Annual General Body Meeting was held on 12th May 1989. The following office bearers were elected for 1989-90:

President	: Shri B. Raghuramaiah
Vice-President	: Dr. P.G. Adsule
Hony. Secretary	: Shri K.L. Sarode
Hony. Jt. Secretary	: Dr. S. Gopalan
Hony. Treasurer	: Shri K.H. Krishnan
Editor	: Shri N. Ibrahim

Shri P.M. Belliappa, I.A.S., Chairman, Tamilnadu Pollution Control Board gave a talk on the Impact of Industrial Pollution on Society and it was follwed by a lecture by Shri C.T. Dwarakanath, Scientist, CFTRI, Mysore on 'Role of micro-organism in human society'.

Pantnagar Chapter

The New Executive Committee members of the chapter, who were unanimously elected in the meeting held on 21st August 1989 were:

President	: Dr. Maharaj Narain
Vice-President	: Dr. Nirankar Nath
Hony. Secretary	: Prof. U.S. Agrawal
Hony. Jt. Secretary	: Dr. B.K. Kumbhar
Hony. Treasurer	: Prof. Dheer Singh

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MANDARIN ORANGE IN INDIA

The Central Food Technological Research Institute (CFTRI), Mysore, has just brought out a Monograph entitled "Mandarin Orange in India - Production, Preservation and Processing".

This booklet covers in brief, aspects on production, propagation, root-stock effects, yield, chemical composition, diseases, post-harvest technologies for fresh fruit handling, storage, packaging, processing as also utilization of by-products. Backed by appropriate references, product specifications and address of equipment manufacture. This monograph is intended for reading by planners, entropreneurs, government organizations, cooperatives, industrial establishments and trade agencies.

Price: Rs.30/- + VPP charges.

Other monographs in the series are: (1) Pineapple - an Industrial Profile (Rs.15/-), (2) Pepper: a Profile (Rs.15/-), (3) Papaya in India (Rs.20/-), (4) Grapes in India (Rs.20/-), (5) Banana in India (Rs.30/-), Monographs on Mango and Guava are expected to be released shortly.

For copies: Please write to the Sales & Distribution Officer, FOSTIS, CFTRI, Mysore - 570 013

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BIBLIOGRAPHY ON BEET MOLASSES

The National Information Centre for Food Science and Technology, (CFTRI), Mysore, has just published an annotated bibliography on BEET MOLASSES covering the period 1959 to 1988. The bibliography lists 94 items.

Price: Rs.50/- + Postage.

Copies can be had from Sales & Distribution Officer, Library, CFTRI, Mysore - 570 013

INSTRUCTIONS TO AUTHORS

1. Manuscripts of papers (in triplicate) should be typewritten in double space on one side of bond paper. They should be complete and in final form. The paper should not have been published or communicated for publication anywhere else. Research Notes should clearly indicate the scope of the investigation and the salient features of the results. Only *invited* review papers will be published.

2. The typescript should be arranged in the following order: Title (to be typed in capital and small letters for Research Papers and all capitals for Research Notes), Authors' names (all capitals) and Affiliation (capitals and small letters). Also give a short running title not exceeding 10 words as a footnote.

3. **Abstract:** The abstract should indicate the principal findings of the paper and typed in single space. It should not be more than 200 words and in such a form that abstracting periodicals can readily use it.

4. Use names of chemical compounds and not their formulae in the text. Methods of sampling, number of replications and relevant statistical analyses should be indicated. Footnotes especially for text should be avoided as far as possible.

5. **Tables:** Tables as well as graphs, both representing the same set of data, should be avoided. Tables should be typed on *separate* sheets. Nil results should be indicated and distinguished clearly from absence of data, which is indicated by '——' sign. Tables should not have more than *nine* columns.

6. **Illustrations:** Graphs and other line drawings should be drawn in Indian ink on tracing paper or white drawing paper preferably art paper not bigger than 20 cm (OY axis) × 16 cm (OX axis). The lettering should be twice the size of the printed letter. Photographs must be on glossy paper and must have good contrast; **three copies** should be sent.

7. **References:** Names of all the authors along with title of the paper should be cited. Abbreviations such as *et al., ibid, idem* should be avoided. References should be serially numbered as superscripts in the order they are cited in the text and the same order should be maintained in the reference list. The titles of all scientific periodicals should be abbreviated in conformity with the World List of Scientific Periodicals, Butterworths Scientific Publication, London, 1962.

   Citation should be as follows (note the underlines also):

   (*a*) *Research Paper*: Jadhav S S and Kulkarni P R, Presser amines in foods, J Fd Sci Technol, 1981, 18, 156.

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

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

   (*d*) *Proceedings, Conferences and Symposia Papers*: Nambudiri E S and Lewis Y S, Cocoa in confectionery, Proceedings of the Symposium on the Status and Prospects of the Confectionery Industry in India, Mysore, May 1979, 27.

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

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

8. Consult the latest issue of the *Journal* for guidance. For "Additional Instructions for Reporting Results of Sensory Analysis" see **issue No. 1** of the Journal.

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