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Nutritive Value of Soy Idli*

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Nutritive value of soy idli was studied using rat growth experiment. Weaning albino rats were fed diet consisting wholely of either unfermented, or fermented soy idli supplemented with lime powder and fenugreek leaves. The control group was fed 20% casein diet. Fermented soy idli was found superior to unfermented soy idli in terms of body weight gain, hepatic protein and vitamin content and femur composition. Supplementation of fermented soy idli with lime and fenugreek leaves further improved the growth, liver and femur composition and was comparable with the control group (20% casein diet) thereby indicating that fermented soy idli along with lime and greens can form a good supplement for undernourished children.

It is well known that legumes form a valuable supplement to cereal diet especially when the diet is lacking in animal proteins[1-4]. Although soybean is superior with regard to nutrient content compared to legumes used in our country, it is not used in any appreciable amounts. The special processing needed to remove the toxic factor and beany flavour presents a problem. In Far East, fermented foods prepared from soybean are popular[5-7]. These foods are mainly fermented by molds which impart a chracteristic flavour not quite familiar to the Indian palate. On the other hand, fermented foods of India such as idli *khaman* and *dhokla* are popular and involve only short and simple process of fermentation.

Previous studies have shown that fermentation brings about changes in the food which improves taste, texture[4-7], nutrient content such as B vitamins[8-14] and also nutritive value and digestibility[6 8,15-19].

Replacement of traditional ingredients with less popular cereals/millets and legumes has been successfully achieved in this laboratory[4,20]. We have been successful in substituting soybean for the traditional black gram in the preparation of idli with improved chemical composition[21]. Present studies were therefore, carried out to determine the nutritive value of fermented soy idli as compared to unfermented soy idli by rat growth experiment. Incorporation of leafy vegetable and lime powder (a mixture of $CaCO_3$, CaO and $Ca(OH)_2$ providing 60% calcium, commonly known as *chuna*) in acid foods such as fermented foods has been shown to give good availability of calcium and vitamin A[20,22]. The effect of such incorporation on nutritive value of soy idli was also studied.

Materials and Methods

The experimental diets were prepared as follows: 1000 g of coarsely ground rice flour, 500 g of finely ground soydal flour and 50 g of common salt were mixed thoroughly with 2500 ml of water. Idlis were prepared by steaming for 5 min in an idli steamer both with unfermented batter and batter fermented at 30°C for 16 hr. One batch of fermented batter was supplemented with 330 g of chopped fenugreek leaves and 5.0 g of lime powder (commercially available *chuna* powder used with betel leaves which is a mixture of $CaCO_3$, CaO and $Ca(OH)_2$). Addition of lime powder did not raise the pH beyond 6.5 and contributed 225 mg calcium/100 g of soy idli mix. The samples were freeze dried. These preparations formed the entire diet without any supplementation. The control diet of 20 per cent casein was prepared as described earlier[23].

Weaning albino rats of Charles Foster strain were used in these studies. Equal number of animals of both the sexes were used in each group. The rats were divided in to six groups and fed following diets *ad libitum* for 4 weeks. (I) 20 per cent casein diet, (II) unfermented soy idli, (III) fermented soy idli, (IV) fermented soy idli supplemented with fenugreek leaves and lime, (V) diet III fed along with group II (Pair-fed) and (VI) diet IV fed along with group II (Pair-fed). Daily food intake and weekly gain in body weight were recorded.

At the end of four week feeding, the animals were

* Part of these studies was presented at 47th Annual Meeting of Society of Biological Chemists (India) at Delhi, October, 1978.

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killed by decapitation under light with ether anesthesia. The abdomen was opened, the liver was removed quickly, freed of adhering blood and connective tissues, weighed and taken for the estimation of protein, thiamin, riboflavin and vitamin A.

Liver protein was determined by the method of Lowry *et al.*[24] and thiamin, riboflavin and niacin by the methods described by the Association of Vitamin Chemists[25] Vitamin A was estimated by the modified method of Gallup and Hoefer[26].

The femurs were removed from both the sides and freed of adhering connective tissues and muscles and used for the analysis of moisture, fat, ash and calcium content by A.O.A.C. methods[27].

Results and Discussion

The data on food intake and body weight gain are given in Table 1. The group fed fermented soy idli showed increased food intake over the group fed unfermented soy idli indicating increased acceptability of the former. The food intake was further increased by incorporation of fenugreek leaves and lime. The rats fed fermented soy idli showed higher body weight gain than those fed unfermented soy idli. The increase in body weight was further improved by incorporation of leafy vegetable and lime. Weight gain per gram protein was higher in rats fed fermented soy idli as compared to that of rats fed unfermented soy idli. Even the pair-fed group showed higher weight gain per gram

TABLE 1. FOOD INTAKE AND BODY WEIGHT GAIN IN RATS FED SOY IDLI DIET

	20% casein (I)	Unfermented soy idli (II)	Fermented soy idli (III)	Fermented soy idli + greens + lime (IV)	diet III + II (V)	diet IV + III (VI)
No. of animals	9	9	8	9	9	9
Protein in diet (g)	20.0	19.5	19.5	19.9	19.5	19.9
Dietary intake/4 Wk						
Food (g)	222.0 ± 4.0	117.0 ± 2.0	177.0 ± 2.0[a]	222.0 ± 3.0	117.0 ± 2.0	117.0 ± 2.0
Energy (Cal)	912.0 ±15.0	426.0 ± 7.0	610.0 ± 6.0[a]	755.0 ± 9.0	403.0 ± 6.0	399 ± 10
Protein (g)	44.0 ± 1.0	23.0 ± 0.4	34.0 ± 0.3[a]	44.0 ± 0.5	23.0 ± 0.4	23.0 ± 0.3
Body Wt. gain* (g)						
for 4 Wk	73.0 ± 3.5	19.0 ± 0.9	40.0 ± 1.7[a]	70.0 ± 2.0	26.0 ± 0.7[a]	33.0 ± 0.8[b]
per 100 Cal.	8.1 ± 0.28	4.6 ± 0.18	6.6 ± 0.23[a]	9.2 ± 0.16	6.2 ± 0.16[a]	8.5 ± 0.21[b]
per g protein	1.67± 0.06	0.87± 0.03	1.19± 0.04[a]	1.60± 0.03	1.11± 0.03[a]	1.47± 0.40[b]

Values are mean±S.E.

*Initial body weight ranged between 45-50 g.

[a]Values significantly different from group II values ($p. < 0.002$)

[b]Values significantly different from group V values ($p < 0.002$)

TABLE 2. LIVER COMPOSITION OF RATS FED SOY IDLI DIET

	20% casein (I)	Unfermented soy idli (II)	Fermented soy idli (III)	Fermented soy idli + greens + lime (IV)	Diet III + II (V)	Diet IV + II (VI)
Final Body wt. (g)	120.00±3.6	66.00±1.5	87.00±1.6[a]	117.00±2.3	73.00±1.3[a]	81.00±1.5
Liver wt. (g)	4.30±0.20	2.10±0.29	3.50±0.12[a]	4.60±0.22	2.69±0.12[a]	3.00±0.08
Protein (g)*	20.80±0.47	14.30±0.47	18.30±0.43[a]	20.10±0.33	16.40±0.25[a]	17.90±0.21[b]
Vitamin A (mg)*	10.10±0.31	4.90±0.16	6.20±0.23[a]	10.60±0.39	5.10±0.21	6.80±0.29[b]
Thiamin (mg)*	1.10±0.055	0.49±0.026	1.05±0.064[a]	1.11±0.088	0.69±0.028[a]	0.79±0.044
Riboflavin (mg)*	3.39±0.12	1.62±0.14	3.11±0.17[a]	3.34±0.26	2.49±0.087[a]	2.51±0.083
Niacin (mg)*	2.57±0.12	1.39±0.063	2.85±0.05[a]	3.11±0.40	2.04±0.067[a]	2.29±0.073

*Values are for 100g liver; values are Mean±S.E.

[a]Values significantly different from group II values ($p < 0.002$)

[b]Values significantly different from group V values ($p < 0.001$)

TABLE 3. FEMUR COMPOSITION OF RATS FED SOY IDLI DIET

	20% casein (I)	Unfermented soy idli (II)	Fermented soy idli (III)	Fermented soy idli + greens + lime (IV)	Diet III + II (V)	Diet IV + III (VI)
Wet wt. (mg)	357.0±6.2	233.0±6.9	278.0±6.6	337.0±9.5	236.0±3.3	278.0±4.7[c]
Dry wt. (mg)	191.0±2.4	114.0±2.1	145.0±2.0	176.0±2.8	129.0±2.2[a]	141.0±1.1[c]
Fat free dry wt. (mg)	182.0±1.9	104.0±2.9	137.0±1.7	167.0±2.6	121.0±2.2[a]	133.0±1.0[c]
Ash wt. (mg)	88.0±4.0	48.0±4.1	71.0±1.1	91.0±2.6	59.0±2.5[a]	70.0±1.2[c]
Calcium (mg)	43.4±0.78	17.5±0.60	26.7±0.37	41.6±0.85	22.0±0.56[b]	27.6±0.84[d]
Calcium (mg 100 g body wt.)	36.2±1.22	26.4±0.67	30.1±0.48	35.4±1.14	30.5±1.08[b]	33.9±1.27[d]

Values are mean±S.E.

Values significantly different from group II values, a is $p<0.001$ and b is $p<0.01$

Values significantly different from group V values, c is $p<0.002$ and d is $p<0.05$

protein than those fed unfermented soy idli. This shows that fermentation improves the nutritive value of soy idli. Similar observations on fermented foods have been made by other investigators[8,16-19]. Supplementation with greens and lime further improves the nutritive value of soy idli. The weight gain per gram protein of soy idli supplemented with greens and lime was comparable with 20 per cent casein diet. The supplementary effects of proteins from greens have been shown by Phansalkar *et al*[28]. Apart from this, the greens also provide other nutrients such as carotene and iron[4]. Children given a supplement of *dhokla* containing greens and lime powder have shown to gain more weight than those receiving *dhokla* without any supplement[20].

Liver weights of rats fed fermented soy idli were higher than those fed unfermented soy idli (Table 2). Liver weight of the rats fed fermented soy idli with greens and lime showed further improvement and was comparable with that of rats fed 20 per cent casein diet. Liver status of the animals as judged by protein, vitamin A, thiamin, riboflavin and niacin contents was better in case of animals fed fermented soy idli. Liver vitamin A of the animals fed fermented soy idli with greens and lime was higher than those receiving diets without such supplement. The availability of vitamin A from greens have been shown earlier[4]. Improved liver status of the rats fed fermented idli and *khaman* has been shown in previous studies in this laboratory[8]. The biochemical status of the pre-school children given supplement of *dhokla* and *dhokla* with greens and lime was much superior to that of controls[20].

Composition of the femur of the rats fed soy idli is given in Table 3. The rats fed fermented soy idli showed better bone status than those fed unfermented soy idli. Incorporation of lime further improves the femur weight, ash and calcium content. This indicates that various nutrients such as calcium, phosphorus and vitamins have good availability in diet supplemented with lime and greens. Incorporation of lime in foods with acidic pH have been achieved successfully in earlier studies in this laboratory[23].

Earlier studies have shown that the fermentation improves the physico-chemical characristics of soy idli[21]. Present studies have further shown that fermentation also imrpoves the biological value as well as the availability of some of the vitamins. Fermented soy idli supplemented with greens and lime can be used as cheap supplement to pre-school children through feeding programmes. Use of such fermented foods, apart from using locally available materials will provide food acceptable to the local palate. Earlier studies have shown that such fermented foods are more acceptable than high protein biscuits[20]. Preliminary feeding trials have shown that fermented soy idli incorporated with lime and leafy vegetables is highly acceptable to pre-school children and is well tolerated by children suffering from protein-calorie malnutrition.

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References

1. Aykroyd, W. R. and Doughty, J., *Legumes in Human Nutrition*, F.A.O., Publication No. 19, 1964.
2. Swaminathanan, M., Availability of Plant Proteins, In Newer Methods of Nutritional Biochemistry (Ed., Albanese, A.A.) Academic Press, New York, 1967, vol. III, 197.
3. Bressani, R., Murillo, B. and Elias, L. G., Whole soybean as a means of increasing protein and calories in maize based diet. *J. Fd Sci.*, 1974, **39**, 197.
4. Rajalakshami, R., Pattern and prevalance of malnutrition. *Baroda J. Nutr.*, 1976, **3**, 1.
5. Hesseltine, C. W., A millennium of fungi, food and fermentation. *Mycologia*, 1965, **57**, 197.
6. Van Veen, A. G., *Fermented Protein Rich Foods*, F.A.O. Report No. FAO/57/3/1966, 1957.
7. Van Veen, A. G. and Steinkraus, K. H., Nutritive value and wholesomeness of fermented foods. *J. agric. Food Chem.*, 1970, **18**, 576.
8. Rajalakshmi, R. and Vanaja, K., Chemical and biological evaluation of effect of fermentation on the nutritive value of foods prepared from rice and gram. *Brit. J. Nutr.*, 1967, **21**, 467.
9. Akinrele, I. A., Fermentation studies on maize during the preparation of a traditional African starch cake food. *J. Sci. Fd Agric.*, 1970, **21**, 619.
10. Van Veen, A. G., Graham, D.C.W. and Steinkraus, K. H., Fermented rice, a food from Eucador. *Arch. Latin Amer. de Nutr.*, 1968, **18**, 363.
11. Van Veen, A. G., Graham, D.C.W. and Steinkraus. K. H., Fermented peanut press cake. *Cereal Sci. Today*, 1968, **13**, 96.
12. Andhah, A. and Muller, H. G., Study on Koko, a fermented food of Ghana. *Ghana J. Agric. Sci.*, 1972, **5**, 95.
13. Quinn, M. R., Beucht, L. R., Miller, J., Young, C. T. and Worthington, R. E., Fungal fermentation of peanut flour. Effect on chemical composition and nutritive value. *J. Fd Sci.*, 1975, **40**, 470.
14. Rajalakshmi, R., Nanavati, K. and Gumastha, A., Effect of cooking procedures on the free and total niacin content of certain foodstuffs. *Indian J. Nutr. dietet.*, 1964, **1**, 276.
15. Hesseltine, C. W. and Wang, H. L., Fermented soybean foods. *Third Internat. Conf. Global Impact of Appl. Microbiol.*, Dec. 7-17, 1969, Bombay.
16. Radhakrishnarao, M. V., Some observations on fermented foods. *Proc. Meeting Protein Needs of Infants and Preschool Children. Internat. Conf.*, Washington DC., Nat. Acad. Sci. Nat Res. Coun. Publ. No. 843, 1968, 291.
17. Platt, B. S., Biological ennoblement: Improvement of the nutritive value of food and dietary regimens by biological agencies. *Food Technol.*, 1964, **18**, 611.
18. Wang, H. L., Ruttle, D. L. and Hesseltine, C. W., Protein quality of wheat and soybean after *Rhizopus oligosporus* fermentation. *J. Nutr.*, 1968, **96**, 109.
19. Morcos, S. R., Hegazi, S. M. and El-Damhoughy, S., Protein nutritive value of bouza and its ingredients. *Ernahr. Wiss.*, 1975, **14**, 34.
20. Rajalakshmi, R. Sail. S. S., Shah, D. G. and Ambady, S. K., The effect of supplements varying in carotene and calcium content on the physical, biochemical and skelatal status of pre-school children. *Brit. J. Nutr.*, 1973, **30**, 77.
21. Ramakrishnan, C. V., Parekh, L. J., Akolkar, P. N., Rao. G. S. and Bhandari, S. D., Studies on soy idli fermentation. *Plant Fd Man.*, 1976, **2**, 15.
22. Rajalakshmi, R. and Ramachandra, K., Calcium incorporation in foodstuffs, In Nutrition and Health (Ed. Kuhanu, J.). Proc. VII Internat. Cong. Nutr. Hamburg, Vol. I, Round Table Discussion II, Peragamon Press, Oxford, 1966, 293.
23. Akolkar, P. N., *Studies on soy idli fermentation.* 1977, Ph.D. Thesis, M. S. University of Baroda, Baroda.
24. Lowry, O. H., Rosebrough, N. J., Farr. A. J. and Randall, R. J., Protein measurement by folin phenol reagent. *J. biol. Chem.*, 1951, **193**, 165.
25. *Methods in Vitamin Assays*, Association of Vitamin Chemists, Interscience Publisher, New York, 1966, 127, 156, 176.
26. Gallup, W. D. and Hoefer, J. A., Determination of vitamin A in liver. *Ind. Engng. Chem., Anal. Ed.*, 1947, **18**, 288.
27. *Official Methods of Analysis*, Association of Agriculture Chemists. Washington DC., 1950.
28. Phansalkar, S. V., Ramachandra, V. and Patwardhan, V. N., Nutritive value of vegetable proteins. I. PER of cereals and pulses and supplementary effect of addition of vegetables. *Indian J. med. Res.*, 1957, **45**, 611.

Comparative Studies on *Atta* (Whole Wheat Flour) and Resultant *Atta* A By-Product of Roller Flour Milling Industry*

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The resultant *atta* from roller flour mills contained lesser protein, ether extractives and ash than the whole wheat flour (*atta*) processed in *chakki* (disc mill). The starch damage in whole wheat *atta* (13.3 to 19.1%) was nearly double than that in resultant *atta* and indicated greater severity of grindling in *chakki*. The chapati water absorption as well as Farinograph water absorption and the dough development time were significantly higher in *atta*. The dough based on resultant *atta* had more resistance to extension and extensibility than the dough based on *atta*. The chapatis made from *atta* were better than those from resultant *atta* with respect to eating quality as well as flavour.

More than 10 per cent of the 36 million tonnes of wheat produced in India is processed in roller flour mills to obtain refined wheat flour (maida) and semolina (soji) as main products and resultant *atta* and bran are obtained as by products. The resultant *atta*, which is also known as 'mill *atta*' in the trade, accounts for 5-15 per cent of the total milled products and contains different streams of fine bran, shorts, clears and tail fines in various proportions. This by product is cheaper than *atta*. Resultant *atta* is generally used for the preparation of chapati in restaurants and industrial canteens, but rarely used in households. Resultant *atta* was found to be superior to *atta* nutritionally and it contains about twice as much thiamin and one and half times as much riboflavin and lysine as compared to whole wheat[1,2]. However, no information is available on the chemical and dough characteristics as well as chapati making quality of resultant *atta*. Results obtained on these aspects are presented in this paper.

Materials and Methods

Samples (J,M,K,C,W, and F) of resultant *atta* and the wheat grist used to obtain the same, were procured from six different roller flour mills situated in different parts of the country having capacities ranging from 30 to 150 metric tons per day. The wheat grist was milled in a *chakki* (disc mill) to obtain *atta*. The samples of both resultant *atta* and *atta* packed in air tight containers were stored in a cold room (4°C) till used for analysis.

Physical characteristics: Bulk density of *atta* and resultant *atta* was determined by measuring the volume of 500 g of *atta* in a measuring cylinder. Sieve analysis was carried out using 200 g *atta* samples in a Buhler plane sifter. The overtailing on different sieves after runnnig the sifter for 5 min. were weighed. The colour of the *atta* samples was measured in a Photovolt Reflectance Meter using tristimulus green filter.

Chemical characteristics: Moisture, ash and ether extractives were determined according to standard AACC methods[3]. Nitrogen content (N) was determined by microkjeldahl method and protein was calculated as N×5.7. AOAC method[4] was used to estimate the free fatty acid (FFA).

Rheological characteristics: Water absorption of *atta* and resultant *atta* for chapati making was measured using Research Water Absorption Meter as per the method standardised by Haridas Rao *et al*[5]. Farinograph characteristics were determined by using Brabender Farinograph[6] at a chapathi dough consistency of 600 BU and at lever position of 1:3. Extensograph characteistics of chapati dough made in Hobart mixer (model N-50) using water equivalent to chapati water absorption, was determined using Brabender extensograph. Only 75 g. of this chapati dough was used for stretching instead of the usual 150 g used for bread dough, since the chapati dough was stiffer. However, the remaining weight was compensated by keeping 75 g weight along with the dough while stretching. The extensograms were drawn at 0 and 1 hr resting periods, and were evaluated as per the standard AACC method[3].

* Presented at the Symposium on 'By Products from Food Industries; Utilization and Disposal' held at Central Food Technological Research Institute, Mysore, on May 29-30, 1980.

Preparation of chapati: Chapati was made from a dough obtained by mixing 100 g *atta* and resultant *atta* with pre-determined quantity of water for 3 min. The dough was rolled into a sheet of 2.0 mm thickness with a wooden roller pin on a specially designed aluminium platform and cut into circular shape of 15 cm diameter. The chapati was then baked (each side) on a hot plate maintained at 400°F for 45 sec. followed by puffing in a gas *tandoor* for 10 sec each side. After cooling, the chapati was evaluated by a panel of 6 judges for its aroma, taste and eating quality. The colour of chapati was measured in a Photovolt reflectance meter using tristimulus green filter.

Results and Discussion

Physical and chemical characteristics of *atta* and resultant *atta* are given in Table 1.

Physical characteristics: Resultant *atta* was more whitish as shown by the higher reflectance value, than the corresponding *atta* which indicates lower bran or higher endosperm content in the resultant *atta*. They were much coarser than *atta* as indicated by the percentage overtailings on a 10××(130μ) sieve. The higher bulk density of the resultant *atta* may be due to its somewhat uniform particle size higher amounts of denser endosperm and lesser bran fraction.

Chemical characteristics: The moisture content in the resultant *atta* was 4-5 per cent higher than the *atta* due to the conditioning of wheat wherein the moisture content of the grain is raised to about 15 per cent. On the contrary, there was a loss of moisture in *atta* during *chakki* milling due to frictional heat development. Higher moisture content in the resultant *atta* may be one of the reasons for its poor keeping quality.

Although there was considerable variation in the ash content of different resultant *atta* samples (0.58-1.39 per cent) the values were still consistently lower than the corresponding *atta* samples. This indicated a lower content of bran in the resultant *atta* samples.

Lower fat content in resultant *atta* indicated the presence of lesser amounts of germ, as most of the fat in wheat kernel is concentrated in germ. The presence of low amount of germ was also substantiated by lower protein content, as germ contained about 3 times the protein as compared to endosperm. Higher FFA in resultant *atta* may be due to higher moisture content, which accelerates the hydrolysis of fat.

Physico-chemical characteristics: The damaged starch content in *atta* was more than double as compared to resultant *atta* (Table 1). This indicated higher severity of grinding in disc type grinder (*chakki*) as compared to roller type mill. As damaged starch has higher water absorption capacity than the natural starch, it is logical to expect higher chapati water absorption for *atta*. It was observed that the chapati water absorption increased with the increase in the damaged starch content.

Farinograph characteristics: The farinograms of different resultant *atta* and the corresponding *atta* (Fig. 1) showed higher dough development time and dough stability for *atta*. The excessively higher dough development time may be due to the presence of higher amount of bran particles in *atta* which may interfere in the quicker development of gluten. Also, preferenctial absorption of water by some constituents other than gluten might also contribute to the longer dough development time.

Extensograph characteristics: The extensibility as

TABLE 1. PHYSICOCHEMICAL CHARACTERISTICS OF RESULTANT *ATTA* (R. *ATTA*) AND *ATTA*

Characteristics*	J		M		K		C		W		F	
	R. *atta*	*Atta*	R. *atta*	*Atta*	R. *atta*	*Atta*	R. *atta*	*Atta*	R. *atta*	*Atta*	R. *atta*	*Atta*
Overtailings of 10X×sieve (%)	71.1	31.3	74.5	23.9	14.7	26.7	78.2	27.7	58.1	27.7	53.4	21.8
Moisture (%)	10.2	8.2	11.1	8.1	9.0	6.0	10.4	7.6	12.4	8.4	13.4	6.6
Ash (%)	0.84	1.55	0.58	1.58	1.24	1.70	1.10	1.50	0.96	1.59	1.39	1.73
Ether extract (%)	1.47	2.10	1.24	1.90	2.12	2.24	1.72	1.96	1.85	3.30	2.46	2.60
Protein (N×5.7) (%)	9.05	9.66	9.05	9.66	9.46	9.92	8.80	9.46	9.27	10.40	10.40	10.65
Free fatty acids (%)	173.6	123.2	210.0	126.0	86.8	81.2	93.2	92.4	78.4	58.8	100.8	67.2
Damaged starch (%)	6.6	14.1	5.9	13.3	10.2	13.9	6.4	19.1	7.4	15.0	6.3	13.7
Chapati water absorption (%)	60.0	69.5	56.5	68.0	61.0	71.0	61.0	72.0	65.0	85.0	60.0	80.0

*Colour values (%) measured in photovolt reflectance meter using tristimulus green filter ranged between 62 and 70 for R. *atta* and 60 and 64 for *Atta*. Similarly, density (g/cc) ranged between 0.49 and 0.58 for R. *atta* and 0.46 and 0.51 for *Atta*.

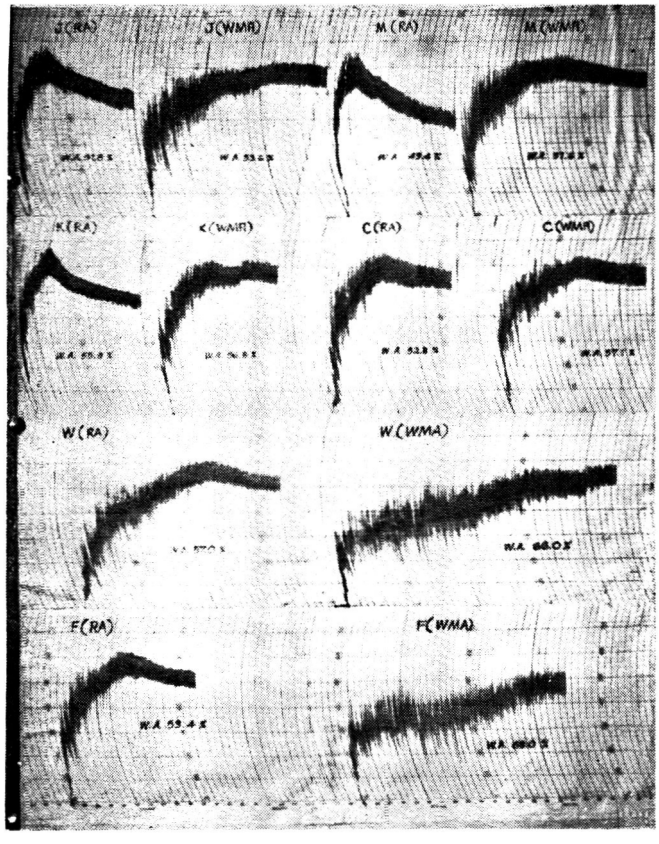

FIG. 1. Farinograms of resultant *atta* (RA) and *atta* (WMA)

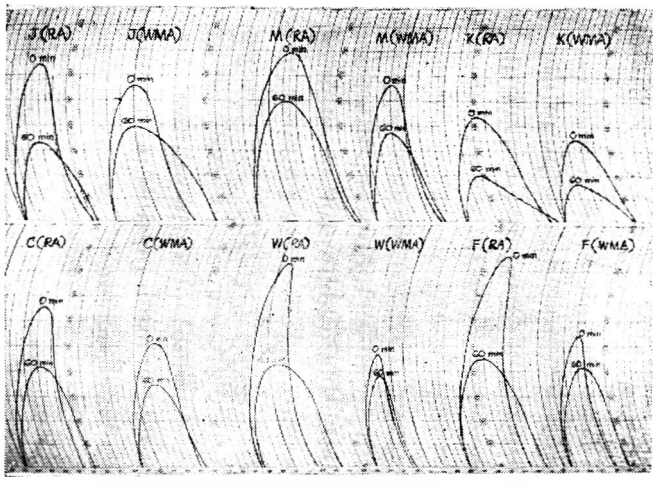

FIG. 2. Extensograms of resultant *atta* (RA) and *atta* (WMA)

well as the resistance to extension were higher for resultant *atta* as compared to *atta* (Fig. 2). This is possibly due to the presence of higher amount of endosperm fraction, wherein all the gluten forming proteins are concentrated. This is also reflected by strength as indicated by the area of the extensograms. *Atta* forms very stiff dough as shown by higher ratio figure. Though the resistance to extension was higher for dough based on resultant *atta*, at 0 hr, rest period, it was of interest to note that on resting for 1 hr, it drastically decreased and was on par with that of *atta* dough, rested for the same period. The higher rate of softening of resultant *atta* during resting was possible due to the presence of high amount of proteolytic enzymes. This indicated that resting the dough made from resultant *atta* improved its rolling characteristics

Chapati making quality: The quality of chapati made from resultant *atta* and the corresponding *atta* are given in Table 2. The chapati made from resultant *atta* was more whitish. The chapati made from resultant *atta* was more leathery and chewy than that made from *atta*. This can be attributed to the presence of higher amounts of gluten as the resultant *atta* contained more of gluten containing endosperm fractions. The extent of leatheriness in chapati made from different resultant *atta* samples depended generally on their ash content. Chapati made from J. M. and W samples of resultant *atta* were more leathery as they had very low bran content which is indicated by their low ash contents.

TABLE 2. QUALITY EVALUATION OF CHAPATI MADE FROM RESULTANT *ATTA* AND *ATTA*.

Characteristics	J		M		K		C		W		F	
	R. *atta*	*Atta*	R. *atta*	*Atta*	R. *atta*	*Atta*	R. *atta*	*Atta*	R. *atta*	*Atta*	R. *atta*	*Atta*
Colour value* (%)	50.0	40.0	57.5	40.5	49.5	41.0	42.0	38.0	45.0	41.5	48.0	44.5
Wheaty aroma	Mild	Good	Mild	Good	Fairly good	Good	Fairly good	Good	Mild	Fairly good	Mild	Fairly good
Chewing Quality	Leathery	Normal	Leathery	Normal	Slightly leathery	Normal	Slightly leathery	Normal	Leathery	Normal	Leathery	Normal
Taste	Bland	Sweet	Bland	Sweet	Slightly sweet	Sweet	Bland	Sweet	Bland	Sweet	Bland	Slightly sweet

*Measured in photovolt reflectance meter using tristimulus green filter.

In contrast chapatis made from K, C and F samples of resultant *atta* were less leathery, as they had comparatively higher bran content. However, very little difference was observed in the eating quality of chapatis made from different *atta* samples showing thereby the similarity in the quality of wheat used in different mills.

The bland taste of chapati made from resultant *atta* as compared to sweet taste of chapati made from *atta* was possibly due to the formation of less sugar during resting of the dough, as it contained lesser amount of damaged starch. Austin and Ram[7] also observed that flour with higher diastatic activity produced sweet chapatis. It is also of interest to note that the typical wheaty aroma observed in chapati made from *atta*, was not found in chapati made from resultant *atta*. The better flavour of chapati made from *atta* is probably due to the development of flavour due to frictional heat produced during grinding in *chakki*.

References

1. Austin, A., *Wheat Research in India*, Indian Council of Agric. Research, New Delhi, 1978, 188.
2. Tara, K. A., Haridas Rao, P. and Bains G. S., Composition and rheological and baking quality of a by-product of Wheat milling simulating whole wheatmeal. *J. Sci. Fd Agric.* 1969, **20**, 368.
3. *Cereal Laboratory Methods*, American Association of Cereal Chemists, St. Paul, Minnesota, USA, 7th Ed., 1962.
4. *Official Method of Analysis*, Association of Official Agricultural Chemists, Washington, D. C., 11th Ed., 1970.
5. Haridas Rao, P. Leelavathi, K. and Shurpalekar, S. R., unpublished data, CFTRI, Mysore, India.
6. Shurpalekar, S. R. and Prabhavathi, C., Brabender farinograph, research extensometer, and Hilliff chapatipress as tools for standardization and objective assessment of chapati dough. *Cereal Chem.*, 1976, **53**, 457.
7. Austin, A. and Ram, A., *Studies on chapati making quality* of wheat. ICAR Bull 31, Delhi, 1971.

Protein Quality of *Chaisathi* (Tea Whitener)

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The protein quality of *chaisathi*, a vegetable-protein based milk, was evaluated against that of low fat milk, skim milk and casein. The protein and fat content of *chaisathi* powder were 35 and 18 per cent respectively. Using growth rate, Protein Efficiency Ratio (PER), Biological Value (BV) and Net Protein Utilization (NPU) as the indicators, the *chaisathi* protein was found comporable with that of low fat milk, skim milk and casein. *Chaisathi* seemed to be a good substitute for dairy milk.

Chaisathi is being produced by the National Dairy Development Board (NDDB) of India to provide less expensive milk to socio economicially poor population. It's current production is 3000L/day and is being sold in low income areas at Baroda. *Chaisathi* contains 10.5 per cent total solids, of which 2.0 per cent is fat. It provides 3.8 per cent protein.

The objective of the present study was to evaluate protein quality of *chaisathi* against protein of low fat milk, skim milk and casein.

Materials and Methods

The samples of *chaisathi*, low fat milk and skim milk powder were obtained from NDDB. Casein was purchased from Amul Dairy, Anand. The protein and fat content of the powders are given in Table 1.

The protein evaluation was based on growth rate, protein efficiency ratio, net protein utilization and biological value. The diets based on different sources of materials contained 10 per cent protein (Table 2). For growth rate and PER[3] determinations, the male weaning rats

TABLE 1. PROTEIN AND FAT CONTENT OF *CHAISATHI* AND MILK BASED SAMPLES

Samples	Protein (%)	Fat (%)
Chaisathi	35	18
Low fat milk	35	18
Skim milk powder	40	<1
Casein	92	<1

weighing between 35 and 40g were divided into 4 groups of 10 each and fed different diets for 28 days.

For BV[3] and NPU[4] determinations, rats weighing between 40 and 50g were divided into 5 groups of 6 each. One group was placed on protein free diet and the remaining groups on experimental diets for a period of 10 days. During the last three days of the experimental period, the rats were placed in metabolic cages to collect urine and fecal material. Urine was collected under toluene. The fecal pellets were collected, air and oven dried and stored until analysis. The rats were ether anaesthetized, the gastrointestinal tract from cardiac to anal end was removed and discarded. The carcasses were weighed, and frozen stored. The urine, fecal and carcass were analysed for nitrogen by microkjeldhal[2] procedure and protein is calculated by N×6.25. The carcasses were pressure cooked and the homogenate was prepared according to the method of Mickelson *et al.*[5] for nitrogen determination. NPU and BV of the protein were calculated.

Student's 't' test was used to find out significant differences between the two means. All tests were considered at 95 per cent of significance[6].

Results and Discussion

The daily food intake and consequently weight gain did not appreciably vary among the groups although casein fed group tended to gain relatively more weight (Table 3). The *chaisathi* fed group was as efficient in food utilization as the low fat milk and casein fed groups. The former tended to be more efficient in food utilization than the skim milk protein fed group(Table3).

The mean value for PER of *chaisathi* protein was comparable to those of low fat milk and skim milk proteins (Table 4). Likewise, the mean values for BV and NPU of different proteins did not vary appreciably. The value for NPU of protein of *chaisathi* was quite comparable with that of casein protein, while the BV of *chaisathi* protein was about 10 per cent lower than that of casein. Based on its PER, NPU and BV, it appears that *chaisathi* could be a good substitute for dairy milk.

TABLE 2. COMPOSITION OF THE EXPERIMENTAL DIETS

Ingredients	Casein (g)	*Chaisathi* (g)	Low fat milk (g)	Skim milk (g)	Protein free (g)
Protein source	11	29.0	29.0	25	0
Vitamin mix[1]	2	2.0	2.0	2	2
Salt mix[2]	4	4.0	4.0	4	4
Groundnut oil	10	9.5	9.5	10	10
Sago	73	55.5	55.5	59	84

TABLE 3. MEANS AND STANDARD ERRORS FOR FOOD INTAKE, GROWTH RATE AND FOOD UTILIZATION

Groups	Food intake (g/day) Mean±S.E.	Wt. gain (g/day) Mean±S.E.	Food intake/wt (g) gain Mean±S.E.
Chaisathi fed	12.05±0.220	3.11±0.187	3.98±0.217
Low fat milk fed	12.14±0.325	3.12±0.146	3.95±0.179
Skim milk fed	13.09±0.242	3.21±0.116	4.50±0.399
Casein fed	12.43±0.347	3.70±0.247	3.44±0.159

TABLE 4. PER, NPU AND BV OF *CHAISATHI* AND OTHER MILK BASED SAMPLES

Source	PER Mean±S.E.	NPU Mean±S.E.	BV Mean±S.E.
Chaisathi	2.58±0.146	81± 4.80	73±2.081
Low fat milk	2.58±0.126	76± 5.406	69±1.995
Skim milk	2.45±0.092	86±11.108	84±3.602
Casein	2.96±0.147	80± 6.929	81±3.921

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References

1. National Academy of Sciences—Nationa Research Council, *Nutrient requirements of laboratory animals*, 2nd Ed. Washington, D.C., 1976 **18.**
2. Oser, L. B., *Hawk's Physiological Chemistry*, McGraw Hill Publishing Co. Ltd., Bombay, 1954.
3. Swaminathan, M., *Essentials of Food and Nutrition*, Ganesh & Co., Madras-17, 1974, **83.**
4. Miller, D. S. and Bender, A.E., The determination of the net utilization of proteins by a shortened method. *Br. J. Nutr.*, 1955, **9,** 382.
5. Mickelson, D., Anderson, A.A. and Bethesda, B. S., A method for preparing intact animals for carcass analysis. *J. Lab. clin. Med.*, 1959, **53,** 282.
6. Ramamurthy, C. G., Viswanathan, K. and Surendran, U.P., *A Concise Book on Statistics*, S. Chand & Co., (Pvt.) Ltd., New Delhi, 1974.

Studies on Pectin Yield and Quality of Some Guava Cultivars in Relation to Cropping Season and Fruit Maturity

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Five guava cultivars ('Banarasi Surkha', 'Allahabad Safeda', 'Tehsildar', Apple Colour' and 'Sardar') were evaluated for pectin contents as affected by the cropping season (rainy and winter) and stage of fruit maturity, (green mature, unripe and half ripe). Significantly higher amounts of crude pectin and jelly units were observed in winter season fruits but the pectin quality in terms of methoxyl content, anhydrogalacturonic acid content, degree of esterification and jelly grade was better in rainy season fruits. Although stage of fruit maturity had no marked effect, yet half ripe fruits yielded more jelly units than unripe ones. 'Banarasi Surkha' and 'Sardar' yielded high grade pectin. but higher number of jelly units were obtained from 'Sardar' and Apple Colour'.

Guava fruit has been found to be a rich source of pectin[1]. Various factors like stage of fruit maturity[1], cropping season[2] and type of cultivar[1-4] affected the quantity and quality of pectin extracted from the tissue. At present, production of pectin in India is quite short of the requirements. Guava is fairly rich in pectic substances and its cultivation has bright scope in the arid zones of North India. Present study was therefore, undertaken to find out the effect of cropping season and appropriate stage of fruit maturity for maximum pectin extraction from important guava cultivars.

Materials and Methods

Five guava cultivars 'Banarasi Surkha', 'Allahabad Safeda,' 'Tehsildar', 'Apple Colour' and 'Sardar' (Syn. 'Lucknow-49') were evaluated for their pectin content. Both rainy and winter season crops during the year 1978–79 were included in the study. Fruits were harvested at two stages: (*i*) Green mature unripe, and (*ii*) half ripe. To ascertain the proper stage of harvest, the firmness of guava fruits was determined with the Magness Taylor pressure type tester, fabricated at the Department of Agricultural Engineering, HAU, Hissar. The fruits with more than 8.5 kg/cm^2 pressure were considered as green mature unripe during both the cropping seasons. Depending on the cultivar, fruits with pressure range of 5.108 to 6.479 kg/cm^2 during rainy season and of 5.460 to 6.628 kg/cm^2 during winter season were considered as half ripe.

Preparation of Sample for Pectin Extraction: After thorough washing and surface drying, the fruits were passed through an electric grater and whole of the crushed material was homogenised. For extraction and isolation, the procedure described by Mc-Cready[5] was adopted with the following details. Freshly grated guava pulp (500 g) was boiled in 1.0 l. water. The pH of the slurry was maintained at 4.0. It was heated for 35 min. at 95-100°C while being stirred continuously. It was then filtered through muslin cloth and immediately cooled below 25°C. The isolation of pectin was done with 70 per cent iso-propanol containing 0.01 N HCl, followed by four washings with 95 per cent iso-propanol. The precipitates of pectin, thus obtained were dried at 35°C in forced draft oven for about 24 hr, till constant weight was obtained. The dried precipitates were weighed and per cent crude pectin yield was calculated. The dried light brown pectin was ground to pass a 60 mesh screen for further analysis.

Characterisation of pectin. The moisture content was determined by drying 1g of pectin at 90°C to a constant weight and the ash content was estimated by ashing in a muffle furnance at 550-600°C for 5 hr.[6] The equivalent weight, methoxyl content and anhydrogalacturonic acid content were determined by the methods of Owens *et al.*[7] Degree of esterification was calculated on the basis of methoxyl and anhydrogalacturonic acid contents[8]. The jelly grade of the pectin was determined by relative viscosity method[9] and the jelly units were calculated by multiplying the jelly grade, with the crude pectin yield obtained from 500 g of fresh guava fruits.

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Results and Discussion

The winter season guavas yielded significantly higher pectin than the rainy season fruits (Table 1), which may be attributed to low moisture content and more compact cells in winter fruits[2]. Presence of higher pectin in winter fruits has also been reported by Sachan *et al*[10] and Gangwar[11]. Although half ripe fruits gave better pectin yields than the unripe ones, the differences were not significant. Different cultivars did not vary significantly in their pectin yields. However, 'Apple Colour' during winter season and 'Tehsildar' during rainy season gave relatively higher pectin yield.

No particular trend was noted regarding moisture and ash contents of the extracted pectin. In general, pectin from winter guavas had higher moisture content than from rainy season (Table 1). The ash content showed no impact of either cropping season or stage of maturity.

Data given in Table 2 reveal that cropping season had no significant effect on the equivalent weight of the pectin. Further, the half ripe fruit yielded pectin with higher equivalent weight, but the differences were not significant. Among the cultivars, 'Banarasi Surkha' and 'Tehsildar' during rainy season and 'Apple Colour' and 'Tehsildar' during winter season gave pectins with the highest equivalent weights.

Pectins from rainy season fruits had significantly higher methoxyl content than those from winter crop (Table 2), but the stage of fruit maturity showed no significant effect. Among the cultivars, 'Banarasi Surkha' during rainy season and 'Apple Colour' during winter season yielded pectins with comparatively higher degree of methoxylation.

The anhydrogalacturonic acid (AUA) content and degree of esterification (DE) which indicate the purity and percentage of total uronide carboxyl groups that are esterified with methanol, respectively, were found to be higher in pectin from rainy season guavas (Table 2). The advancement of maturity had no significant effect on AUA or DE, although the former, showed a decreasing trend. The pectin from 'Banarasi Surkha' contained the highest AUA content and also the DE.

Irrespective of all the above factors, rainy season fruits yielded pectins with significantly higher jelly grade over winter season fruits (Table 3). The half ripe fruits yielded slightly higher grade pectin than the unripe ones. Among cultivars, 'Banarasi Surkha' and 'Sardar' yielded high grade pectins, while 'Tehsildar' had the lowest grade pectin. The jelly grade was found to be inversely proportional to the crude pectin yield(Table 1), but it was directly proportional to the methoxyl content (Table 2) which is an index of esterification and an

TABLE 1. EFFECT OF CROPPING SEASON AND STAGE OF FRUIT MATURITY ON CRUDE PECTIN YIELD, MOISTURE AND ASH CONTENTS OF PECTIN IN VARIOUS GUAVA CULTIVARS

Cultivar	Crop	Crude pectin yield (%)			Moisture (%)			Ash (%)		
		Green mature unripe	Half ripe	Mean	Green mature unripe	Half ripe	Mean	Green mature unripe	Half ripe	Mean
Banarasi	Rainy	0.71	0.77	0.74	10.7	9.4	10.1	1.2	1.1	1.2
Surkha	Winter	0.79	1.03	0.91	12.7	12.4	12.6	1.3	1.6	1.5
Allahabad	Rainy	0.74	0.77	0.76	13.4	10.5	12.0	1.2	1.1	1.2
Safeda	Winter	0.93	0.96	0.95	14.9	12.2	13.6	1.7	1.9	1.8
Tehsildar	Rainy	0.66	0.91	0.79	9.8	10.1	10.0	1.2	1.4	1.3
	Winter	0.97	1.00	0.99	11.8	10.4	11.1	1.3	1.6	1.5
Apple	Rainy	0.69	0.87	0.78	14.4	12.4	13.4	1.7	1.9	1.8
Colour	Winter	1.08	1.34	1.21	12.3	14.7	13.5	1.6	1.8	1.7
Sardar	Rainy	0.74	0.79	0.77	12.1	11.7	11.9	1.4	1.7	1.6
	Winter	0.90	1.05	0.98	10.2	11.0	10.6	1.5	1.8	1.7
Mean	Rainy	0.71	0.82	0.77	12.1	10.8	11.5	1.3	1.4	1.4
	Winter	0.93	1.08	1.01	12.4	12.1	12.3	1.5	1.7	1.6

't' at 5%: Difference between stages of maturity is not significant for crude pectin yield, moisture and ash. Difference between seasons is significant for crude pectin yield only.

TABLE 2. EFFECT OF CROPPING SEASON AND STAGE OF FRUIT MATURITY ON BIOCHEMICAL CHARACTERS OF GUAVA PECTIN

Cultivar	Crop	Equivalent weight			Methoxyl (Me0%) (Ash–H_2O–free basis)			Anhydrogalacturonic acid (%)			Degree of esterification (%)		
		Green mature unripe	Half ripe	Mean	Green mature unripe	Half ripe	Mean	Green mature unripe	Half ripe	Mean	Green mature unripe	Half ripe	Mean
Banarasi	Rainy	834.5	830.1	832.3	7.69	7.52	7.61	69.24	70.40	69.82	63.11	60.95	62.03
Surkha	Winter	674.3	740.0	707.2	5.83	6.08	5.96	59.02	61.43	60.23	56.10	56.13	56.12
Allahabad	Rainy	763.3	715.1	739.2	6.51	6.61	6.56	62.18	66.21	64.20	59.32	56.67	58.00
Safeda	Winter	700.5	720.3	710.4	6.46	5.06	5.76	64.07	56.25	60.16	57.22	51.09	54.16
Tehsildar	Rainy	747.8	837.5	792.7	5.76	6.15	5.95	59.34	59.01	59.18	55.08	59.17	57.13
	Winter	829.8	810.7	820.3	5.63	6.14	5.89	56.31	59.70	58.01	56.76	58.35	57.56
Apple	Rainy	639.8	785.0	712.4	7.50	6.20	6.85	72.26	59.70	65.98	58.90	58.89	58.90
Colour	Winter	740.0	851.0	795.5	6.14	6.19	6.17	61.77	59.02	60.40	56.39	59.54	57.97
Sardar	Rainy	646.8	713.3	680.1	7.62	6.73	7.18	72.64	65.87	69.26	59.53	57.96	58.75
	Winter	756.4	767.4	761.9	5.70	5.43	5.57	58.67	56.97	57.82	55.10	54.17	54.64
Mean	Rainy	726.4	776.2	751.3	7.02	6.64	6.83	67.13	64.24	65.69	59.19	58.73	58.96
	Winter	740.2	777.9	759.1	5.95	5.78	5.87	59.97	58.67	59.32	56.31	55.86	56.09
't' at 5%	Stages	Not significant			Not significant			Not significant			Not significant		
	Seasons	Not significant			Significant			Significant			Significant		

TABLE 3. EFFECT OF CROPPING SEASON AND STAGE OF FRUIT MATURITY ON JELLY GRADE AND JELLY UNITS OF GUAVA PECTIN

Cultivar	Crop	Jelly grade			Jelly units/500g fruit)		
		Green mature unripe	Half ripe	Mean	Green mature unripe	Half ripe	Mean
Banarasi Surkha	Rainy	184	206	195	650	778	714
	Winter	157	159	158	622	817	720
Allahabad Safeda	Rainy	176	162	169	655	623	639
	Winter	146	151	149	666	723	695
Tehsildar	Rainy	152	158	155	501	721	611
	Winter	144	147	146	704	734	719
Apple Colour	Rainy	189	172	181	651	744	698
	Winter	129	135	132	697	906	802
Sardar	Rainy	190	200	195	697	795	746
	Winter	147	149	148	662	780	721
Mean	Rainy	178	180	179	631	732	682
	Winter	145	148	147	670	792	731
't' at 5%	Stages	Not significant			Rainy season : Not singificant		
	Seasons	Significant			Winter season : Significant		
					Not significant		

important factor in controlling the setting time of jellies.

Winter season fruits yielded more jelly units than the rainy season ones. In rainy season, the stage of fruit maturity did not significantly affect the jelly units, whereas in winter crop, the half ripe fruits yielded significantly higher jelly units than the unripe ones. Among the cultivars, 'Sardar' yielded pectins with the highest jelly units in rainy season, whereas, in winter season, the highest number of jelly units were recorded in 'Apple Colour', followed by 'Sardar'. The lowest jelly units in both the cropping seasons were obtained in pectin from. 'Tehsildar', Although the pectin from 'Banarasi Surkha' had the highest jelly grade, yet due to lesser pectin yields, the jelly units were comparatively lower than those from 'Apple Colour' guavas.

From these results, it is clear that the rainy season crop y elded pectin with high jelly grade and higher degree of methoxylation, but due to low yields, the jelly units were lower while winter crop pectin had low jelly grade and lower methoxyl content. Due to higher pectin yields, the number of jelly units was found higher. Although the stage of fruit maturity had no significant effect, yet half ripe fruits gave more pectin yield. Among the cultivars, 'Apple Colour', 'Sardar' and 'Tehsildar' were found promising with respect to pectin yield and jelly units.

References

1. Pruthi, J. S., Mookerji, K. K. and Girdhari Lal, A study of factors affecting the recovery and quality of pectin from guava. *Indian Fd Pckr*, 1960, **14**, 7.
2. Rathore, D. S., Effect of season on the growth and chemical composition of guava fruits. *J. Hort. Sci.*, 1976, **51**, 41.
3. Pal, D. K. and Selvaraj, Y., Changes in pectin and PE activity in developing guava fruits. *J. Fd Sci. Technol.*, 1979, **16**, 115.
4. Verma, A. R. and Srivastava, J. C., Pectin in guava during growth and maturity. *Indian J. Hort.*, 1965, **22**, 318.
5. McCready, R. M., in *Methods in Food Analysis*, Joslyn, M.A. (Ed.) Academic Press, Inc., New York. 1970, 565.
6. Ranganna, S., *Manual of Analysis of Fruit and Vegetable Products*, TMH Publishing Co. Ltd., New Delhi, 1977, 21.
7. Owens, H. S., Mc Cready, R. M., Shepherd, A. D., Schultz, S.H., Pippen, E. L., Swenson, H. A., Miers, J. C., Erlandsen R. F. and Maclay, W. D., Methods used at Western Regional Research Laboratory for extraction and analysis of pectin materials, AIC-340, Western Regional Research Laboratory, Albany, California, 1952.
8. Schultz, T. H., Determination of the ester methoxyl content of pectin by saponification and titration; determination of AUA content by decarboxylation and titration of liberated carbon dioxide. *Methods Carbohydrate Chem.*, 1965, **5**, 189.
9. Rouse, A. H., Evaluation of pectins from Florida's citrus peels and cores. *Citrus Ind.*, 1967, **48**, 9.
10. Sachan, B. P., Pandey, D. and Shanker G., Influence of weather on the chemical composition of guava fruits var. 'Allahabad Safeda'. *Punjab Hort. J.*, 1969, **9**, 119.
11. Gangwar, B. M., Biochemical studies on growth and ripening of guava. *Indian Fd Pckr*, 1972, **26**, 13.

Penetration of Bacteria into Muscles of Goat, Pork and Poultry Meat

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Penetration of four pathogenic bacteria into goat, pork and poultry meat was studied. ***Bacillus cereus*** **and** ***Staphylococcus aureus*** **caused linear proteolysis, while** ***Salmonella typhimurium*** **penetrated along the perimyseal septa in goat muscle. In pork muscle,** ***B. cereus*** **and** ***Escherichia coli*** **showed linear proteolysis, while** ***S. aureus*** **and** ***S. typhimurium*** **produced extensive liquifaction along the peri-and endomyseal septa. In poultry muscle, proteolysis occurred along peri-and endomyseal septa by** ***B. cereus***, ***S. aureus*** **and** ***E. coli***.

The spoilage of meat by proteolytic bacteria is important not only from meat hygiene, but also from public health viewpoint as these bacteria involved in the process are usually pathogenic or toxigenic in nature. Bottom *et al.*[1] and Hesengawa *et al.*[2] observed that muscle protein breakdown occurred during microbial

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spoilage of meat. Tarrant *et al.*[3] had shown that the onset of spoilage in pork by *P. fragi* coincided with extracellular proteolytic activity and an increased amount of ultrastructure damage to muscle fragments. In pure culture study in beef meat, Dainty *et al.*[4] reported that proteolytic spoilage was mainly due to gram negative bacteria. Penetration of bacteria into meat is caused by proteolytic species only. Thus non-proteolytic bacteria did not invade even when associated with proteolytic organisms.[5]

The present investigation has been aimed to determine the extent to which food poisoning bacteria invade the deeper layers of different types of meat. This study is expected to strengthen the understanding of the spoilage process of meat.

Materials and Methods

Cultures: The bacterial cultures used were *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimurium*. These cultures were procured from Central Research Institute, Kasauli (India). All cultures were maintained on nutrient agar slants and were grown on nutrient agar plates.

Collection of samples: The meat samples were collected aseptically from the slaughter house between 6 and 7 a.m. and analysed within 2 hr. The meat samples were normal and pink in colour.

Penetration of bacteria: The muscles were cut into strips of meat $2\times2\times5$ cm in size in case of goat and pork and $2\times2\times4$ cm in case of poultry. Meat samples were obtained from thigh portions of the goat and pork and sternal muscles of the poultry. They were dipped in alcohol to remove the surface microflora[6]. The meat strips were immersed in 3 per cent molten agar containing 0.1 per cent mercuric chloride to inhibit the growth of surface contaminants. The solid agar was removed from one end of the meat strip and inoculated with the bacteria. Two strips of meat were inoculated at the same time for each experiment. These inoculated strips of meat were hooked to the pins hanging in a stand placed in the jar. This ensured the incubation of the meat strips vertically. The jar containing the strips was placed at 37°C until the organisms had shown sufficient growth, i.e. for 20 hr in case of goat and poultry meat for all the types of bacteria studied; 44 hr in case of pork for *B. cereus* and *S. aureus* and 20 hr for *E. coli* and *S. typhimurium*. The trials were conducted in duplicate.

After the incubation, one strip each of the meat inoculated with different bacteria was placed in a tube containing sufficient amount of 10 per cent formalin-saline. The tube was placed in the water bath at 56°C for 30 min. to fix the tissue. Pieces of 0.3 cm were removed from the inoculated end from a distance of 2, 3 and 4 cm of the strips in case of goat and pork and 2 and 3 cm in case of poultry meat. The sections were cut by embedding in paraffin wax, stained with hemotoxylin eosin stain.

Another strip of meat was cut at 2, 3 and 4 cm from the inoculated end and streaked on the selective media and incubated at 37°C for 24-28 hr. Colonies from the plates showing growth were further examined to confirm the identity of the organism.

Selective media: Mannitol egg-yolk phenol polymyxin agar for *B. cereus*, *Staphylococcus* medium 110 for *S. aureus*, brilliant green agar for *E. coli* and *Salmonella-Shigella* agar and desoxycholate citrate agar for *S. typhmiurium* were used.

Results

Goat muscle strips: *B. cereus* and *S. aureus* penetrated each to a depth of 3 cm producing linear proteolysis by liquefying the muscle fibres and their surrounding septa, whereas *S. typhimurium* penetrated along the perimyseal septa to a depth of 4 cm. *E coli* did not show any indication of penetration (Table 1, Fig. 1a and 1b). The muscle strips were incubated for 20 hr when the organism had attained maximum growth.

TABLE 1. PENETRATION OF VARIOUS BACTERIA IN MUSCLES OF DIFFERENT SPECIES OF MEAT ANIMALS

Source of meat	Bacteria	Incubation period (hr)	Depth of penetration* (cm)	Type of proteolysis
Goat	*B. cereus*	20	3	Linear
	S. aureus	20	3	Linear
	E. coli	20	—	No proteolysis
	S. typhimurium	20	4	Along muscular septa
Poultry	*B. cereus*	24	3	Along muscular septa
	S. aureus	24	3	Around the muscle fibres
	E. coli	24	3	Along muscular septa in branching manner & in muscle bundles
	S. typhimurium	24	3	Along muscular septa and linear
Pork	*B. cereus*	44	4	Linear
	S. aureus	44	4	Around muscle fibres
	E. coli	20	4	Linear
	S. typhimurium	20	4	Linear

*As evidenced by proteolysis and culturing.

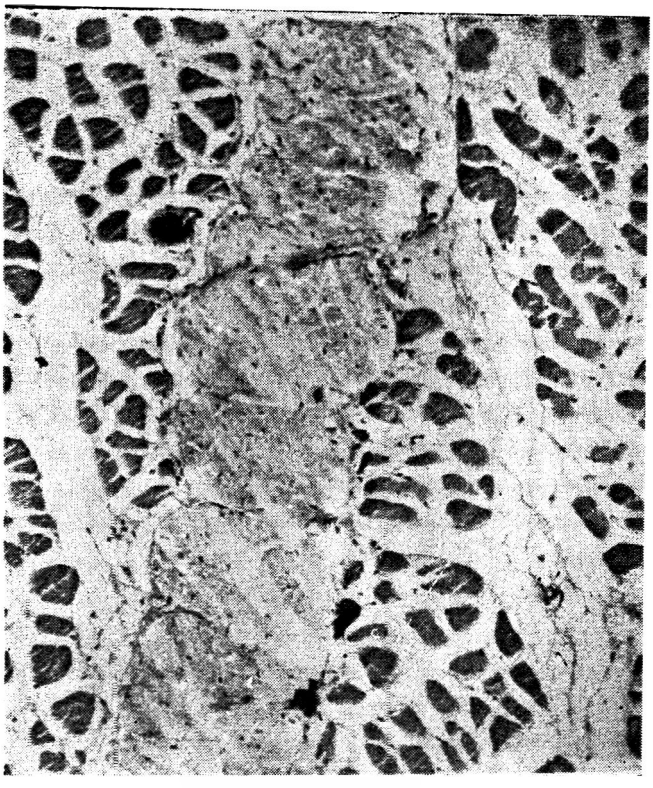

FIG. 1*a*. Goat Muscle strip infected with *B. cereus:* linear proteolysis H. E. X 100.

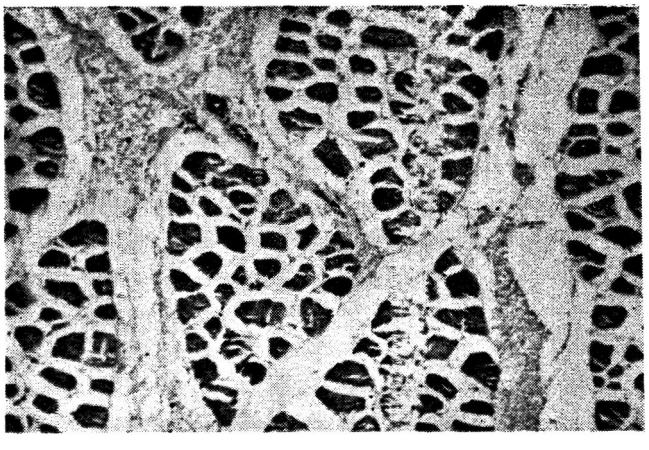

FIG. 1*b*. Goat muscle strip infected with *S. typhimurium:* proteolysis along the muscular septa H. E. X 100.

Pork muscle strips: All the organisms showed a penetration upto 4 cm (Table 1, Fig. 2). Maximum growth was shown by *B. cereus* and *S. aureus* in 44 hr and *E. coli* and *S. typhimurium* in 20 hr. *B. cereus* and *E. coli* produced linear proteolysis dissolving the muscle fibres as well as the encircling endomyseal septa, whereas *S. aureus* and *S. typhimurium* produced extensive liquifaction along the peri-and endomyseal septa usually sparing the muscle fibres.

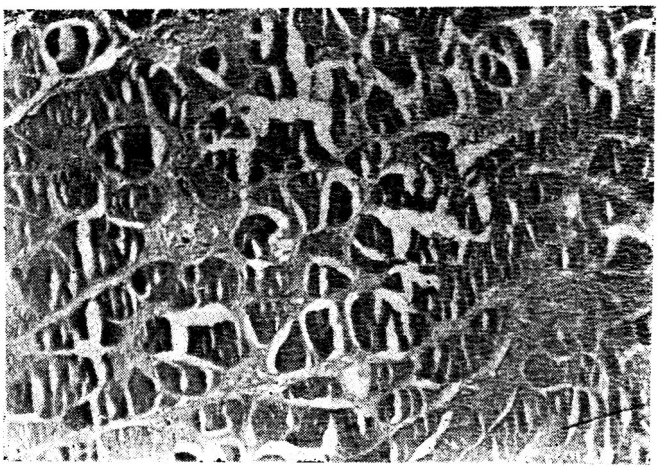

FIG. 2. Pork muscle strip infected with *S. aureus:* proteolysis around the muscle fibres H, E. X 100.

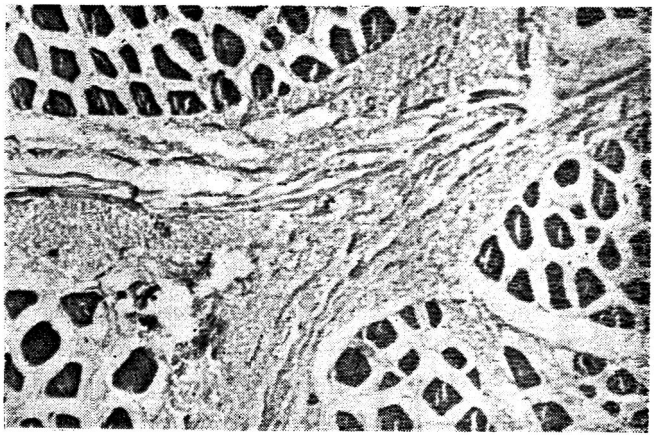

FIG. 3. Poultry muscle strip infected with *B. cereus:* proteolysis along muscular septa and extending around the individual muscle fibres H. E. X 100.

Poultry muscle strips: Each of these organisms penetrated to a distance of 3 cm along the muscular septa within 24 hr (Table 1 Fig. 3). The proteolysis in *B. cereus, S. aureus* and *E. coli* extended along the peri- and endomyseal septa, whereas in *S. typhimurium* the proteolysis was noticed only along the perimyseal septa.

Discussion

The penetration of meat by bacteria results from the breakdown of the muscle fibres as well as the associated connective tissue in the interstitial septae. The pattern of proteolysis varied depending upon the type as well as the animal species from which the muscle strips were derived.

In goat muscle strip, the linear proteolysis caused by *B. cereus* and *S. aureus* as compared to the proteolysis by *S. typhimurium* along the perimyseal septa indicate

that the former two organisms had comparatively more proteolytic activity as the linear penetration occurs due to the dissolution of muscle fibres as well as the peri-and endomysium. In pork muscle, the linear liquefaction by *B. cereus* and *E. coli* as compared to the perimyseal dissolution in case of other two organisms likewise indicate high degree of lytic activity in case of the former two organisms. In poultry strips, the proteolysis by *B. cereus*, *S. aureus* and *E. coli* extended along the peri- and endomyseal, whereas it was only along the perimyseal septa in *S. typhimurium* suggesting the higher proteolytic activity in case of former three organisms. The deeper penetration in case of pork as compared to goat muscle indicated, that the pork muscle fibres were easily penetrable which might be due to relatively soft nature of the muscle fibres. The strip of the poultry muscle was only 3 cm in length and the penetration of the organism was observed throughout the strip, the chances of deeper penetration in the event of the use of the longer strip could not be ruled out. The variation in the penetration of bacteria in muscles of goat, poultry and pork reflected variation in the nature and extent of production of proteases. This could possibly be due to the variable nutritional conditions provided by muscle strips of different species. The penetration had been reported to occur only due to proteases and the non-proteolytic species did not invade, even when they were present along with proteolytic species. This was probably because the penetration originated in the area of growth of a microcolony of the proteolytic species and thus non-proteolytic bacteria were excluded[5].

References

1. Bottom, R. J., Bralder, L. J. and Price, J. F., Effects of four species of bacteria on protein muscle 1. Protein solubility and emulsifying capacity. *J. Fd. Sci.*, 1970, **35**, 799.
2. Hesengawa, T. A., Pearson, A. M., Price, J. F. and Lechowich R. V., Action of bacterial growth on the sarcopalsmic and urea soluble proteins from muscle. Effect of *Clostridium perfringens*, *Salmonella enteridis*, *Achromobacter liquifaciens*, *Streptococcus faecalis* and *Kurthia zepfii*. *Appl. Microbiol.*, 1970, **20**, 117.
3. Tarrant, P.V.J., Jenkins, A. M., Pearson, A. M. and Duston, T. R., Proteolytic enzyme preparation from *P. fragi*: Its action on pig muscle. *Appl. Microbiol.*, 1973, **25**, 976.
4. Dainty, R. H., Shaw, B. G., Deboer, K. A. and Scheps, E.S.J., Protein changes caused by bacterial growth of beef. *J. appl. Bacteriol.*, 1975, **39**, 73.
5. Gill, C. O. and Penny, N., Penetration of bacteria into meat. *Appl. Environ, Microbiol.*, 1977, **33**, 1284.
6. Lepovetsky, B. C., Weiser, H. H. and Deathrage, F. E., A microbiological study of lymph nodes, bone marrow and muscle tissue obtained from slaughtered cattle. *Appl. Microbiol.*, 1953, **1**, 57.

Fractionation of Wood Phenolics and Their Use in Brandy

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Phenolics were extracted from Indian oak (*Quercus* sp.), teak (*Tectona grandis*), red cedar (*Cedrella toona*) and sandal (*Santanum album*) by ethanol. Parameters like ratio of wood to ethanol, concentration of solvent, and time of contact for extraction of phenolics were studied. Extracted phenolics were segregated into, flavanoids and non-flavanoids as well as soluble and insoluble fractions in solvents like chloroform, ethylacetate and amyl alcohol. The fractionated phenolics were estimated after removal of the solvents. Studies on organoleptic evaluation of young brandy on addition of these fractions of phenolics (free from solvents) have indicated that some combinations of fractions reduce the harshness of the product.

It is an age old practice to store wine and distilled liquor such as brandy, whisky and rum in wooden casks. The type of woods used for casks are specific and they impart desirable characteristics to the product. The changes that take place during storage or "ageing" or "maturation" as it is called, is not very well understood. Apart from the interaction among the different constituents of the content, the phenolics extracted from wood have an important role in the formation of compounds which are directly responsible for the changes. Singleton[1] used ethanol extract and chips of oak wood to impart oak flavour to wine. Development of aromatic flavour in brandy treated with Indian oak wood chips was reported by Venkataramu *et al*[2]. In the present study, data on fractionation of phenolics from four Indian woods with different solvents in single stage and

also sequentially extracted, along with extraction of flavanoids, and non-flavanoids and phenolics extracted by other solvents are presented. Effect of addition of some of these extracted phenolics on the taste of brandy is also recorded.

Materials and Methods

Teak (*Tectona grandis*) and red cedar (*Cedrella toona*) woods were obtained from local timber yard. Deoiled sandal wood (*Santanum album*) was obtained from Sandal Wood Oil Factory, Mysore. Oak wood (*Quercus* species) was obtained from Forest Department of Himachal Pradesh. These woods were powdered and used.

Distilled solvents such as ethanol, ethyl acetate, chloroform, n-butanol and amyl alcohol and AR grade formaldehyde, gallic acid and trichloroacetic acid were used.

Total phenolics were estimated using colorimetric procedure of Singleton and Rossi[3] using spectronic 20 (gallic acid was used as standard). Flavanoids and non-flavanoids were determined by the procedure of Kremling and Singleton[4].

Extraction of phenolics was done using different concentrations of ethanol by stationary and under agitation (Emenvee rotary shaker, 230 r.p.m) and samples were drawn at intervals of 24 hr for analysis. In case of sequential extraction, wood powder was completely submerged in ethanol and every 24 hr, the supernatant was removed and was replaced by fresh ethanol. Addition of formaldehyde and estimation of phenolics in the supernatant gave the value for non-flavanoids. The difference between the total flavanoids and non-flavanoids was computed and the content of flavanoids was calculated. In case of immisible solvent system, the phenolics in different solvents were estimated after separating them.

Results and Discussion

The quantity of phenolics extracted by 45-60 per cent ethanol varied from 4 to 6 per cent by weight of powdered Indian oak, teak, red cedar and sandal woods, at each intervals of 24 hr, under agitation (Table 1).

The quantity of phenolics extracted per gram of wood was highest in red cedar followed by Indian oak, sandal and teak. In red cedar, the ethanol extractable phenolics formed nearly 28 per cent by weight of wood. This appears to be on the high side. It is not known whether this high value is due to any artefact introduced by any other constituent of wood which possess a similar reaction as phenolics with the reagent. The extraction of phenolics appeared to be almost complete even at the end of the first 24 hr, as there was no significant increase in the further extraction at the end of 72 hr.

Singleton *et al.*[5] have suggested that flavanoids content in wine matured in wooden casks can be a measure of maturation. Differentiation of flavanoids from non-flavanoids based on their solubility in formaldehyde is one of the recognised methods. This method was used to measure the formaldehyde soluble (non-flavanoids) and formaldehyde insoluble (flavanoids) in these woods.

The results presented in Table 2 indicate that the red cedar contained a high amount of flavanoids followed

TABLE 1. PHENOLICS EXTRACTED (MG/G DRY WT. BASIS) FROM DIFFERENT WOODS AFTER SOAKING FOR DIFFERENT PERIODS IN ETHANOL

Concn. of wood (%)	Concn. of ethyl alcohol (%)	Indian oak			Teak			Red cedar*		Sandal		
		24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	72 hr	24 hr	48 hr	72 hr
4	45	146.5	146.5	155.0	24.9	23.5	23.5	269.2	271.9	56.75	61.25	67.50
,,	50	182.5	182.5	197.5	25.0	26.0	24.5	261.0	271.9	53.75	58.75	66.25
,,	55	210.0	210.0	210.0	30.5	26.5	26.0	269.2	271.9	61.25	73.75	67.50
,,	60	146.5	146.5	153.7	29.0	27.5	26.0	264.8	271.9	58.75	58.75	67.50
5	45	158.0	158.0	158.0	24.0	24.4	23.6	215.3	282.7	53.00	54.00	56.00
,,	50	182.0	182.0	188.0	25.6	25.2	23.6	215.3	282.7	53.00	54.00	60.00
,,	55	124.0	124.0	136.0	26.0	26.0	24.4	226.2	261.0	56.00	58.00	64.00
,,	60	152.0	158.0	164.0	26.0	26.0	25.6	212.1	261.0	56.00	58.00	64.00
6	45	128.3	—	—	22.0	23.0	22.6	199.0	262.8	48.33	56.66	57.49
,,	50	93.3	100.0	103.3	23.3	23.3	23.3	199.0	253.7	56.66	57.49	59.99
,,	55	121.6	146.6	126.6	25.6	25.5	24.3	189.8	262.8	53.33	56.66	57.49
,,	60	150.0	155.0	155.0	25.6	24.5	24.6	195.9	271.1	54.99	59.99	59.99

*Not estimated for 48 hr.

TABLE 2. FLAVANOIDS AND NON-FLAVANOIDS PRESENT IN INDIAN OAK, TEAK, RED CEDAR AND SANDAL WOODS*

Type of Wood	1st extract			2nd extract			3rd extract		
	Initial phenolics (mg/g of wood)	Flavanoids (mg)	Non Flavanoids (mg)	Initial phenolics (mg/g of wood)	Flavanoids (mg)	Non Flavanoids (mg)	Initial phenolics (mg/g of wood)	Flavanoids (mg)	Non Flavanoids (mg)
Indian oak	195.0	189.0	6.0	63.0	58.1	5.0	6.8	5.5	1.3
Teak	27.0	19.7	7.3	26.0	21.1	4.9	2.8	2.4	0.4
Red cedar	271.9	263.7	8.1	111.6	107.1	4.4	29.7	28.5	1.2
Sandal	60.0	57.3	2.7	10.4	8.2	2.2	—	—	—

*50% Ethanol extraction after 24 hr.

TABLE 3. FRACTIONATION OF ETHANOL SOLUBLE PHENOLICS USING CHLOROFORM, AMYL ALCOHOL AND ETHYL ACETATE

Type of wood	Ethanol soluble phenolics (mg/g of wood)	Chloroform		Amyl alcohol		Ethyl acetate	
		Soluble (mg)	Insoluble (mg)	Soluble (mg)	Inscluble (mg)	Soluble (mg)	Insoluble (mg)
Indian oak	182.0	23.8	152.6	141.2	37.9	54.9	122.4
Teak	25.6	5.0	15.4	17.1	6.7	9.8	14.3
Red cedar	272.7	23.9	246.5	171.6	99.7	139.3	127.2
Sandal	56.0	22.1	28.2	36.5	17.1	28.2	23.3

by Indian oak, sandal and teak as observed earlier. These data indicate that flavanoid content in red cedar was significantly high compared to the other woods analysed. Sequential extraction simulates conditions of extraction that would occur in reused barrels. The trend in concentration of flavanoids indicates that repeated use of barrels may extend the period of ageing. From economic considerations it would seem preferable to transfer liquor in a few days from a new barrel to an used one; or the residence time of contents be controlled by frequent analysis to allow dissolution of desirable level of phenolics.

The ethanol extractable phenolics of four woods were fractionated using different solvents such as chloroform, amyl alcohol, and ethyl acetate. The concentration of soluble and insoluble phenolics of these woods in these solvents are presented in Table 3.

The data indicate a general trend that the chloroform insoluble is more than soluble phenolics. Aqueous soluble phenolics is more in sandal followed by teak, Indian oak and lowest being in red cedar. The trend reverses when amyl alcohol is used, where solvent solubles are more compared to insolubles. Aqueous soluble phenolics was highest in Indian oak followed by teak, sandal and lowest in red cedar. In case of ethyl acetate the aqueous layer had high phenolics in Indian oak and teak, while phenolics in solvent layer was high when red cedar and sandal woods were used.

The phenolic fractions of the same wood either singly or in combination were added to fresh/green brandy and subjected to sensory evaluation along with controls. Results indicated that combination of phenolic fractions of the wood when added improved the taste of the brandy.

References

1. Singleton, V. L., in *Chemistry of Wine Making*, A. D. Webb, (Ed.) American Chemical Society, Washington, 1974, 254.
2. Venkataramu, K., Patel, J. D. and Subba Rao, M. S., Studies on extraction of phenolics of wood by brandy. *Indian Fd Pckr.*, 1982, **34**, 22.
3. Singleton, V. L., and Rossi, J. A., Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.*, 1965, **16**, 144.
4. Kremling, T. E. and Singleton, V. L., An estimation of the non-flavonoid phenols in wine. *Am. J. Enol. Vitic.*, 1969, **20**, 86.
5. Singleton, V. L., Suliman, A. R., and Kramer, C., An analysis of wine to indicate ageing in wood on treatment with wood chips. *Am. J. Enol. Vitic.*, 1971, **22**, 161.

The Microbiological Quality of Ice Creams Sold in Hyderabad City

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The bacteriological quality of ice cream and ice fruits sold in Hyderabad were assessed. Three hundred and one samples were collected from hotels, restaurants, ice cream parlours, and local vendors. These were assessed for standard plate count (SPC) coliforms by MPN method and for pathogens. Only 58 (19.4%) were within ISI specifications for SPC and coliform counts. *Staphylococcus aureus* was isolated from 20.3% samples, and *Escherichia colli* from 7% samples. The slab ice cream sold in hotels was superior to other varieties sold by local vendors.

Currently available data suggest that the foods sold in Indian markets are of very poor quality[1-3]. Ice cream is a popular milk product consumed by people of all categories. The present study was undertaken to assess the microbiological quality of the ice creams sold in Hyderabad city (India).

Materials and Methods

Three hundred and one samples of ice creams consisting of slabs (31), cups (70), bars (25), and lollies (150) were collected in sterile, covered containers from hotels, restaurants, parlours and local vendors. They were brought to the laboratory in ice flasks and immediately analysed.

Analysis: The samples were allowed to thaw and graded dilutions were prepared using Ringer's solution. The total counts were estimated by the standard method[4]. The coliforms were estimated by the most probable number method using MacConkey's broth[5]. Gas positive tubes were streaked on EMB agar and colonies showing greenish metallic sheen were further confirmed by biochemical reactions as *Escherichia coli.*

Staphylococci were isolated by inoculating the sample into mannitol-salt broth for enrichment, followed by streaking on milk-salt agar for isolation. The *Staphylococci* strains obtained thus were tested for their coagulase activity and confirmed as *S. aureus.*

Salmonella was tested according to the method of Thatcher and Clark[6]. Modified bile agar medium was used for the isolation of the vibrios, after processing through alkaline peptone water for enrichment[7]. Potassium tellurite medium was used for isolation of diphtheria bacillus.

The enterococci were isolated by inoculating the sample into SF medium and then streaking on sodium azide medium.

The identification of isolated organisms, were confirmed by biochemical reactions,[8,9] wherever necessary.

Results and Discussion

The ISI has prescribed SPC of not more than 250000/g and coliform count of not more than 100/g. and the ice cream should be free from pathogenic organisms[10]. Out of the 301 samples studied, only 58 (19.4 per cent) were within the standard limits (Table 1). There was a definite correlation between the cost of the ice cream and its sanitary quality. The costlier slab ice creams (77.4 per cent) sold in the hotels were within the standard limits, when standard plate counts and coliform counts were considered together. Among the other varieties, only 12.9 per cent of the cup ice creams, 24 per cent of bar ice creams, 9 per cent of the cones, and 10.7 per cent of the lollies were within the limit specified by ISI.

Of the samples analysed, 88.7 per cent were positive for coliforms. The coliforms isolated included *Klebsiella, Enterobacter cloaca, E. aerogenes* and *E. hafniae.* However, only 19.3 per cent had counts higher than ISI specifications. *Escherichia coli* was found in 7 per cent of the samples.

Enterococci were isolated from 44.7 per cent of the samples. Since raw milk and dairy products contain *Enterococci* as contaminants, their presence alone does not indicate the sanitary history of the product, unless associated with other indicator bacteria.

Staphylococcus aureus was the only pathogen isolated. This was more frequently isolated from the cheaper varieties of ice creams where 20.3 per cent of the samples were positive for the pathogen (Table 2).

TABLE 1. STANDARD PLATE COUNTS AND COLIFORM COUNTS IN ICE CREAM SAMPLES

Ice cream type	Standard plate counts			Coliform counts		No. within ISI
	Samples with $<25\times10/^4$g	Samples with $>25\times10^4$/g	Average count (log)	Samples with <100/g	Samples with >100/g	
Slab	25 (80.6)	6 (19.4)	5.106	29 (93.5)	2 (6.5)	24 (77.4)
Cups	10 (14.3)	60 (85.7)	5.597	64 (91.4)	6 (8.6)	9 (12.9)
Chacobars	9 (36.0)	16 (64.0)	5.545	21 (84.0)	4 (16.0)	6 (24.0)
Cones	5 (20.0)	20 (80.0)	6.572	18 (72.0)	7 (28.0)	3 (9.0)
Lolly	20 (13.3)	130 (86.7)	5.793	111 (74.0)	39 (26.0)	16 (10.7)
Total	69 (22.2)	232 (77.8)		243 (80.7)	58 (19.3)	58 (19.3)

Figures in the parenthesis indicate the percentages.

TABLE 2. PATHOGENS ISOLATED FROM THE ICE CREAM

Ice cream type	Samples collected	*Staphylococcus aureus*		*Escherichia coli*	
		No. of samples	% samples	No. of samples	% samples
Slab	31	5	16.1	1	3.2
Cups	70	14	20.0	1	1.4
Cones	25	5	20.0	1	4.0
Chacobar	25	12	48.0	2	8.0
Lolly	150	25	16.8	16	10.7

The poor quality of the cups, cones and wrappers used is probably one of the factors contributing to the heavy load of organisms. Repeated handling and unhygienic sorroundings is another factor for the poor quality of the cheap, locally sold ice creams. Small scale manufacturers produce low grade ice creams of substandard quality. The consumption of these ice creams is an important source of infection.

Acknowledgement

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References

1. Vijaya Rao, D., Bhagirati, B. and Gopal Rao, K. R., Incidence of drug resistant coliforms in some ready to serve foods. *J. Fd Sci. Technol.*, 1978. **15**, 201.
2. Sherikar, A. A., Ajinkya, S. M., Khot, J. B. and Sherikar, A. T., The microbial flora of ready-to-cook pork products-a public health point of view. *J. Fd Sci. Technol.*, 1979, **16**, 228.
3. Guha, A. K., Das. H. N., Roy, R. and Dewan, M. L., Microorganisms in ice creams and their public health significance. *J. Fd Sci. Technol.*, 1979, **16**, 161.
4. Walter, W. G., *Standard Methods for the Examination of Dairy Products*, American public Health Association., Inc., New York, 1967.
5. *International Standard for Drinking Water*, World Health Organisation. Geneva, 2nd Ed., 1963.
6. Thatcher, F. S. and Clark, D. S., *Microorganisms in Foods: Their Significance and Methods of Enumeration*, University of Toronto Press, Canada, 1973, 148.
7. *Bacteriological Analytical Manual.*, Food and Drug Administration, Washington, DC., 4th Ed., 1973.
8. Baiely, R. W. and Scott, E. G., *Diagnostic Microbiology*, The C. V. Mosby Comp. 14th Ed., 1976.
9. Buchannan, R. E. and Gibbons, N. E., *Bergey's Manual of Determinative Bacteriology*, Williams and Wilkins Company Baltimore, 8th Ed, 1975.
10. *Code for Hygienic Condition for Manufacture, Storage and Sales of Ice creams, IS*-5839, Indian Standards Institution, New Delhi.

Studies on Sunflower Oil with Reference to its Keeping Quality

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Ten sunflower oil samples of five different varieties from irrigated and rainfed trials were studied for their keeping quality. Samples were withdrawn at every thirtieth day and different chemical tests were carried out. Different varieties behaved differently. The keeping quality of the oils obtained from crop grown under rainfed conditions was better than those obtained from irrigated condition.

Sunflower oil is valued highly as a source of polyunsaturated fatty acids. It has a high level (60-72 per cent) of the essential fatty acid, linoleic acid[1]. As the magnitude of unsaturation is more in sunflower oil, it is susceptible for rapid autooxidation during storage. Chapmna *et al*[2]. have reported that the fatty acid composition of oil varies with environment. Oils obtained from plants grown under different environmental conditions may show different storage behaviour. Studies have been made on the storage of watermelon seed oil and muskmelon seed oil. Studies have shown that crude groundnut oil stores well in glass, aluminium and tinned-brass containers. But literature on studies conducted on the storage quality of sunflower oil is scarce under Indian conditions. Yousuf Alikhan *et al*[3]. have studied the storage of bulk quantities of raw sunflower and groundnut oils. Storage study in small glass containers (as may be useful for household purposes) of crude sunflower oil samples extracted from seeds grown under different climatic conditions was taken up and the results are reported.

Materials and Methods

Ten sunflower oil samples obtained from five different varieties ('EC 68414', 'EC 68415' SuF1, 'Su F2' and 'K 2') grown under irrigated and rainfed conditions were used for this study. The oil was extracted from sun-dried seeds by Soxhlet fat extraction using solvent ether[4]. A jet of nitrogen gas was passed through the oils to remove any other matter present. Samples of oil were stored in small screw capped colourless bottles 3/4 th height filled. Oil was withdrawn every thirtieth day and the following analyses were carried out. Lipid hydroperoxides which are the first compounds formed during the course of rancidity were measured in terms of the peroxide value[5] and results expressed as ml of 0.02N sodium thiosulphate. The volatile carbonyl compounds formed were measured by thiobarbituric acid value[6] at 400 nm and by anisidine value[7] at 420 nm. The free fatty acids were measured using a rapid colorimetric method[8]. The oxirane compounds were measured by picric acid test[9].

Results and Discussion

At 180th day, all samples had developed off-flavour which was confirmed by nine untrained judges. The toxic character of rancid oil has been studied[10].

Peroxide value: The initial peroxide values were found to be higher in irrigated samples than in rainfed samples as shown in Table 1. Several studies have indicated that the levels of unsaturated fatty acids and oils in soybeans are strongly influenced by the environment[2,11,12,13]. There was significant increase in peroxide value between the initial period and 180 days showing the O_2 uptake by the oil. Although 'peroxides' are possibly not directly responsible for the taste and odour of rancid fats, their concentration as represented by the peroxide value is often useful in assessing the extent to which spoilage has advanced[5]. Among the five different varieties, the variety, 'SuF_1,' had the highest mean peroxide value and the variety 'K_2' the lowest with significant difference between them. The greater the magnitude of unsaturation, the greater is the liability of fat to undergo oxidative rancidity[5]. The mean peroxide values were significantly higher or same in all varieties except for 'SuF'_1, under irrigated than under rainfed conditions. Different varieties showed different peroxide values.

Free fatty acid contents: The initial FFA contents were also found to be higher for irrigated samples than for the rainfed samples as shown in Table 2. Increase in FFA during storage could be noticed as reported by Cox and Pearson[5]. As rancidity is usually accompanied by free fatty acid formation, the determination is often

TABLE 1. PEROXIDE VALUE* OF SUNFLOWER OIL COLLECTED FROM CROPS GROWN UNDER IRRIGATED AND RAINFED CONDITIONS

Storage period (days)	Irrigated					Rainfed				
	EC.68414	EC.68415	SuF1	SuF2	K2	EC.68414	EC.68415	SuF1	SuF2	K2
0	3.25	2.1	1.8	1.9	2.2	1.3	0.85	1.15	1.15	0.60
30	3.50	3.0	2.0	2.5	3.0	5.6	3.00	5.50	3.40	2.00
60	4.00	4.0	2.7	3.5	3.8	5.9	3.90	5.60	3.80	2.80
90	4.00	4.4	3.4	4.6	4.2	6.1	6.90	10.00	6.70	3.45
120	6.45	10.3	7.9	7.8	6.7	6.3	6.95	10.05	7.50	4.10
150	7.00	10.4	9.2	9.1	8.5	6.4	7.70	10.30	8.10	4.25
180	7.50	10.6	9.8	9.1	8.7	6.7	8.10	14.80	11.30	5.05
Mean	5.10	6.39	5.26	5.51	5.31	5.47	5.34	8.20	5.99	3.18

*ml of 0.02N sodium thiosulphate/g of oil

TABLE 2. FREE FATTY ACID CONTENT (% OLEIC ACID EQUIVALENT) OF SUNFLOWER OIL COLLECTED FROM CROPS GROWN UNDER IRRIGATED AND RAINFED CONDITIONS

Storage period (days)	Irrigated					Rainfed				
	EC.68414	EC.68415	SuF1	SuF2	K2	EC.68414	EC.68415	SuF1	SuF2	K2
0	1.24	2.09	0.96	1.12	1.50	0.71	1.70	0.76	1.02	1.30
30	1.95	2.65	1.41	1.36	2.16	1.12	2.23	1.05	1.53	2.44
60	5.49	4.93	1.59	1.53	3.82	2.09	3.71	1.72	2.89	4.47
90	10.80	10.62	2.75	3.23	7.64	2.41	3.71	1.81	2.89	4.47
120	10.97	11.00	2.82	3.40	7.64	2.57	3.71	2.00	2.89	4.55
150	11.68	11.22	2.99	3.51	7.64	2.74	3.82	2.00	2.89	4.55
180	11.68	11.22	3.35	3.57	7.81	2.74	4.35	2.10	2.97	4.61

TABLE 3. THIOBARBITURIC ACID VALUE (O.D. AT 400 NM) OF SUNFLOWER OIL COLLECTED FROM CROPS GROWN UNDER IRRIGATED AND RAINFED CONDITIONS

Storage period (days)	Irrigated					Rainfed				
	EC.68414	EC.68415	SuF1	SuF2	K2	EC.68414	EC.68415	SuF1	SuF2	K2
0	0.03	0.06	0.11	0.09	0.11	0.02	0.12	0.05	0.03	0.05
30	0.10	0.13	0.15	0.02	0.11	0.6	0.15	0.10	0.09	0.10
60	0.15	0.18	0.21	0.17	0.17	0.18	0.36	0.50	0.27	0.14
90	0.19	0.31	0.23	0.25	0.40	0.20	0.42	0.51	0.44	0.18
120	0.19	0.35	0.35	0.41	0.23	0.46	0.46	0.56	0.58	0.35
150	0.35	0.50	0.36	0.55	0.56	0.29	0.49	0.65	0.61	0.36
180	0.38	0.63	0.36	0.62	0.59	0.34	0.54	0.68	0.65	0.40

used as a general indication of the condition and edible quality of oils[5]. The samples grown under irrigated condition showed significantly higher FFA contents than rainfed samples at the time of the development of off-flavour. The different varieties showed different FFA values. It has been reported in cotton seed, that both variety and location significantly influence the moisture and FFA contents. The higher FFA content in the irrigated

samples may be due to the higher moisture content available. Several studies have indicated that in soybeans the lipoxygenase activity appears to be genetically controlled[2,11,12,13].

Thiobarbituric acid value (TBA): The volatile carbonyl compounds show up in the TBA value[14]. Significant increase in TBA values could be noticed between the initial and final periods of storage indicating the development of off-flavour (Table 3). The TBA values were found to be low in the samples analysed. The main volatile carbonyl compounds resulting from the autoxidation of linoleic acid are hexanal and 2,4-decadienal[15]. The concentration which could be very important in the formation of rancid off-flavour are very small even in a highly autooxidized sample[14] both in irrigated and rainfed grown samples.

Anisidine value: The anisidine value signals the presence of carboxyl compounds[14]. Upto 90th day, the values were zero except for three samples (Table 4). The mean anisidine value of irrigated samples were found to be higher than rainfed samples. Varietal difference could also be noticed. Except for 'EC-68415', the mean anisidine values of the other varieties were significantly higher or same in irrigated than in rainfed conditions.

Picric acid value: Increase in picric acid value during the storage was observed, which indicated the development of rancidity (Table 5). The values were low as has been indicated by Maza[16]. Varietal difference could be noticed. No significant difference could be noticed in oils obtained from irrigated and rainfed crops. The oil samples were grouped into irrigated and rainfed and the discriminant function was worked out with the five different chemical tests. The mean values of the results of the different chemical tests of both irrigated and rainfed samples are given in Table 6. The discriminant function for this is as follows:

$$z=(X_1) + (1.52X_2) - (16.17X_3) + (39.17X_4) - (243.08X_5)$$ where,

$X_1X_2X_3,X_4$ and X_5 stand for peroxide value, free fatty acid content, thiobarbituric acid value, anisidine value and picric acid value respectively.

TABLE 4. ANISIDINE VALUE (O.D. AT 420 NM) OF SUNFLOWER OIL COLLECTED FROM CROPS GROWN UNDER IRRIGATED AND RAINFED CONDITIONS

Storage period (days)	Irrigated					Rainfed				
	EC.68414	EC.68415	SuF1	SuF2	K2	EC.68414	EC.68415	SuF1	SuF2	K2
90	0.013	0	0	0	0	0.034	0	0	0	0.035
120	0.038	0	0.076	0.213	0.099	0.056	0.051	0.022	0.099	0.046
150	0.038	0	0.087	0.235	0.099	0.056	0.081	0.058	0.133	0.046
180	0.038	0.024	0.099	0.255	0.099	0.056	0.164	0.096	0.175	0.049
Mean	0.038	0.008	0.087	0.234	0.099	0.056	0.099	0.59	0.136	0.047

Anisidine value was zero in all varieties under irrigated and rainfed conditions upto 60th day of storage.

TABLE 5. PICRIC ACID VALUE (O.D. AT 490 NM) OF SUNFLOWER OIL COLLECTED FROM CROPS GROWN UNDER IRRIGATED AND RAINFED CONDITIONS

Storage period (days)	Irrigated					Rainfed				
	EC.68414	EC.68415	SuF1	SuF2	K2	EC.68414	EC.68415	SuF1	SuF2	K2
60	0	0	0	0	0	0.013	0.027	0.026	0.006	0.016
90	0.029	0.003	0.019	0	0	0.033	0.036	0.042	0.015	0.018
120	0.046	0.039	0.030	0.030	0.029	0.033	0.036	0.042	0.022	0.018
150	0.046	0.040	0.35	0.031	0.05	0.033	0.038	0.042	0.024	0.018
180	0.050	0.046	0.037	0.031	0.038	0.033	0.038	0.042	0.035	0.018

TABLE 6. MEAN VALUES OF DIFFERENT CHEMICAL TESTS OF IRRIGATED AND RAINFED SAMPLES

	Irrigated	Rainfed
Peroxide value	5.540	5.637
Free fatty acid content	5.120	2.640
Thiobarbituric acid value	0.280	0.320
Anisidine value	0.093	0.079
Picric acid value	0.031	0.031

The discrimination is found to be highly significant (P=0.01), indicating that the parameters studied were closely associated with the development of rancidty of the oil.

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References

1. Langstraat, A. and Jurgens, B. V., Characteristics and composition of vegetable oil bearing materials. *J. Am. oil Chemists Soc.*, 1976, **53**, 241.
2. Chapman, G. W., Robertson, J. A. and Burdick, D., Fatty acid composition affected by environment. *J. Am. oil chemists Soc.*, 1976, **53**, 54.
3. Yousuf Ali Khan, A., Lakshminarayana, T., Azeemoddin, G., Atchyuta Ramayya, D. and Thirumala Rao, S. D., Shelf life of sunflower oil and groundnut oil. *J. Fd Sci. Technol.*, 1979, **16**, 90.
4. *Official methods of analysis of the Association of Official Analytical Chemists* A.O.A.C., Washington, 1975, 12th edn., No. 7, 045, 135.
5. Cox, H. E. and David Pearson. *The Chemical Analysis of Foods*, Chemical publishing Co. Inc., New York, 1962, 1st American Eds, 421.
6. Dobbs, J. W., Unpleasant odous of rapeseed oil heated to frying temperatures. 1975 M. Sc. thesis, University of of Manitoba.
7. Jirousora, J., Modified determination of the anisidine value in oxidised fats and oils. *Nahrung.*, 1975, **19**, 319.
8. Lowry, R. R. and Tinsley, I. J., Rapid colorimetric determination of free fatty acids. *J. Am. oil chemists Soc.*, 1976. **53**, 470.
9. Fioriti, J. A., Benty, A. P. and Sims, R. J., The reaction of picric acid with Epoxides-I. A colorimetric method. *J. Am. oil chemists Soc.*, 1966, **43**, 37.
10. Kajimoto, G., Yoshida, H. and Shibahara, A., Toxic character of rancid oil. XVI. Comparison of the nutritive value between thermally oxidised oil and tocopherol free fresh oil. *Yukagaku.*, 1975, **24**, 511.
11. Hammond, E. G., Fehr, W. R. and Snyder, H. E., Improving soybean quality by plant breeding. *J. Am. oil chemists Soc.*, 1972, **49**, 33.
12. Howell, R. W. and Collins, F. I., Variability of linolenic and linoleic acids in soybean oil. *Agron. J.*, 1957, **49**, 593.
13. Hitchock, C. and Nichols, D. W., In *Experimental Botany*, Plant Lipid Biochemistry (Sutchiff & Mablberg, F., Eds), Academic press. London & New York, 1971, Vol. 4, 152.
14. Basile Tsoukalas and Werer Grosch. Analysis of fat deterioration-comparison of some photometric tests., *J. Am. oil chemists Soc.*, 1977, **54**, 490.
15. Kimoto, W. I. and Gaddis, A. M., Precursors of Alk-2, 4-dienals in autooxidised Lard. *J. Am. oil chemists' Soc.*, 1969, **46**, 403.
16. Maza, M. P. and Vioque, E., Micro determination of epoxy acids in oils. *Grasas Aceites.*, 1975, **26**, 78.

Organochlorine Pesticide Residues in Groundnut Oil

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Samples of groundnut oil were randomly collected from the markets in Lucknow and Sitapur District of Uttar Pradesh, India. Organochlorine pesticide residues were estimated by gas-liquid chromatography using an electron capture detector (^3H). Besides DDT, its metabolites DDD and DDE, aldrin and isomers of HCH were detected in significantly high levels in almost all the samples analyzed. A comparison of DDT levels in groundnut oil with that earlier reported in India suggests, a steady state with regard to DDT contamination of food stuffs.

The presence of persistent organochlorine pesticide (OCP) residues, mainly 2, 2-bis (parachlorophenyl)-1, 1, 1-trichloroethane (DDT) and 1,2,3,4,5,6-hexachlorocyclohexane (HCH) in feed stuffs and body tissues in India, is now well accepted. Main source of these environmental toxicants in body tissues appears to be the contamination of food chain. Organochlorine insecticides are lipophilic in nature and therefore, an assessment of fatty foodstuffs for the contamination of pesticide residues is of relevance. Contamination of various oils with DDT and BHC in India has already been reported[1,2]. Our earlier studies have also shown the presence of DDT, BHC and Aldrin in mustard, coconut and vegetable oils[3]. Groundnut is an important oil seed and is consumed as kernels or as oil. It is usually attacked by termites and white-grubs in Punjab and other places, and therefore, aldrin and BHC etc. are recommended for the control of these pests[4]. Present study was designed to assess the pesticide contamination of groundnut oil collected randomly from two districts of Uttar Pradesh.

Materials and Methods

Sixty samples of oil from the local market (40 from Lucknow and adjoining areas and 20 from Sitapur and adjoining areas) were collected randomly, during September to November, 1980, and stored at room temperature till analysed, which is generally within 48 hr.

Chemicals used were of Analar grade unless otherwise indicated and checked against ECD (electron capture detector) contamination. n-Hexane used for extraction was purified and tested for pesticide contamination. Other solvents used were double distilled. Care was also taken to avoid glassware contamination by pesticides.

The method used for extraction of pesticides was that of Mills *et al*[5]. n-Hexane was first treated with 1 ml of distilled water in a clean test tube and placed in a liquid air-methanol bath to remove the traces of acetonitrile, if any. The unfrozen phase, that of hexane, was further treated with conc. H_2SO_4 (1 ml) three times, to remove the fat and the cleaned hexane was collected.

Cleaned samples were then analysed by gas-liquid chromatograph, Varian aerograph series "2400" with $3H^+$ detector, under the following operating conditions[6].

Carrier gas	..	Purified nitrogen passing through silica gel & molecular sieve to remove moisture & oxygen respectively.
Gas pressure	..	65 p.s.i.
Flow rate	..	40 ml/min
Injector temp.	..	190°C
Column temp.	..	180°C
Detector temp.	..	200°C
Attenuation	..	x^{16}, x^4
Current	..	10^{-9} mA.
Column	..	Glass spiral column length 6ft., internal dia 1/8" packed with gas chrome Q (80/100 mesh) coated with 1.5 per cent OV-17+1.95 per cent OV-210 by weight.
Sample	..	1.0 – 5.0 μl

Standards used were obtained from Poly Science Corporation, Illinois (U.S.A.). Residues detected were further confirmed by thin layer chromatography.

Results and Discussion

Levels of aldrin, HCH, DDT and its metabolites detected in the samples are given in Table 1. A compa-

TABLE 1. ORGANOCHLORINE INSECTICIDE RESIDUES (P.P.M.) IN GROUNDNUT OIL SAMPLES FROM LUCKNOW AND SITAPUR DISTRICTS.

Pesticides	Sitapur Mean±S.E.	Lucknow Mean±S.E.
BHC (HCH)	1.306±0.135	1.340±0.319
Aldrin	0.892±0.255	0.290±0.056
p,p'—DDE	1.330±0.178	0.634±0.136
p,p'—DDD	1.158±0.243	0.367±0.051
p,p'—DDT	0.384±0.077	0.858±0.270
ΣDDT	2.960±0.396	1.956±0.370

HCH includes α, β and γ-isomers (lindane).
ΣDDT—Total DDT equivalent.

rison of the present findings has been made with those of earlier ones in Table 2.

Residue level of HCH given in Table 1. comprises of alpha, beta and gamma-isomers, the main stereoisomers of hexachlorocyclohexane (HCH). Although the range of contamination of groundnut oil with HCH is more in the samples collected from Lucknow region (0.199-6.421 ppm) than in Sitapur region (0.296-2.360 ppm), there is not much variation in the mean residue levels (1.340 and 1.300 ppm respectively).

Residues of aldrin, in Sitapur district were about 4 times the levels found in Lucknow. It may be mentioned here that aldrin is closely associated with dieldrin, an epoxidation product of aldrin, but it could not be detected as our analytical procedure in the study involved treatment of extracted samples with concentrated H_2SO_4 which precludes dieldrin[7]. However, since tolerance limit fixed by the F.D.A. of the U.S.A. for aldrin/dieldrin in oil and oil seeds is 0.00 ppm, the exclusion of dieldrin has no impact on the study.

TABLE 2 DDT RESIDUES IN OILS

Commodity	Place	No. of samples examined	Residue level (ppm)
Groundnut	Delhi[1]	5	5 - 7.1
,,	Hyderabad[2]	10	—
Mustard	Delhi[1]	3	22.1 - 25.7
,,	Lucknow[3]	25	1.4 - 10.9
Sesame	Delhi[1]	3	10.0 - 12.1
Coconut	Delhi[1]	3	9.3 - 10.6
,,	Lucknow[3]	25	0.3 - 1.6
Vegetable	,,	25	0.55 - 5.4

Superscripts indicate reference numbers

Total DDT equivalent which represents rather complete exposure of crop for DDT was also higher in Sitapur (2.960 p.p.m.) than in Lucknow (1.955 ppm). In Sitapur, the total DDT equivalent was contributed by 1.330 ppm of p,p'-DDE and 1.158 ppm of p,p'-DDD (TDE). Level of p,p'-DDT was only 0.384 ppm. However, in Lucknow 1.95 ppm of total DDT equivalent was contributed by 0.634 ppm of p,p'-DDE and 0.366 ppm of p'p'-DDD. The parent compound p.p'-DDT was 0.857 ppm. Therefore, it may be concluded that either the rate of DDT metabolism in groundnut crop of Sitapur was higher than Lucknow or there was chronic exposure.

The suggested daily intake[8] of fat in the form of vegetable oils like groundnut, sunflower, etc. is 15g which led to about 45 μg of total DDT equivalent 19 μg of HCH and 13 ppm of aldrin, as the daily intake in Sitapur, and about 30 ppm of DDT, 19 ppm of HCH and 4.5 ppm of aldrin in Lucknow. Since pesticidies enter into the body mainly through food chain contamination, edible oils may be taken as one of the main sources of pesticide buildup in the body tissues of humans. The possibility indicated by Thakare *et al*[1]. that the oilseeds and oil are the sources of contamination of human fat therefore, seems to be correct.

If we compare the present residue level in the groundnut oil with that of the work done about 8 years ago in Delhi[1] and Hyderabad,[2] the residue level range of DDT seems to be in a steady state.

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References

1. Thakare, S.K., Dewan, R. S. and Gulati, K.C., Build up of DDT in human fat—oils and oilseeds as possible source. *Pesticides.*, 1969, 3 (2), 13.
2. Lakshminarayana, V. and Krishna Menon, P., Exposure of general population in Hyderabad to DDT. *J. Fd Sci. Technol.*, 1972, 9, 82.
3. Siddiqui, M.K.J., Seth, T. D. and Saxena, M.C., Pesticide pollution in India. III. organochlorine pesticides in edible oils., *Pesti ides.*, 1980 (Accepted).
4. Udeaan, A. S. and Deshmukh, S. N., Residues of Aldrin, BHC and chlordane in ground nut kernels and oil. *Proceedings of the First All India Symposium on Progress and Problems in Pesticide Residue Analysis* held at Ludhiana on 10th-19th November, 1971, 140.

5. Mills, P. A., Collaborative study of certain chlorinated pesticides in dairy products. *J. Ass., off.agric. Chem.*, 1961, **44**, 171.
6. Siddiqui, M.K.J., Saxena, M. C., Seth, T. D., Bhargava, A.K., Krishna Murti, C. R. and Kutty, D., Chlorinated hydrocarbons pesticides in the blood of new born babies in India. *Pestic. Monit. J.*, In press.
7. Skaftason Johannes, F. and Johernesson, T., Organochlorine compounds in Icelandic animal body fat and butter fat: local and global sources of contamination. *Acta Pharmacol et Toxicol.*, 1979, **44**, 156.
8. Gopalan, C. and Narasinga Rao, B. S. *Dietary allowances for Indians*, Indian Council of Medical Research, New Delhi.

Effect of Methyl Parathion on Body Weight, Water Content and Ionic Changes in the Teleost, *Tilapia Mossambica* (Peters)

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***Tilapia mossambica* fish were exposed to sublethal concentration (0.09 ppm) of methyl parathion for 48 hr. The Changes in body weight and water content were not significant. The sodium, potassium and calcium ions decreased in all the tissues of methyl parathion exposed fishes. These results suggest that the regulation of osmotic balance is more effective through the operation of salt pump rather than water pump.**

The indiscriminate use of pesticides can be considered as one of the factors which alters the environment, causing several imbalances in the ecosystem, especially to the denizens of the aquatic media[1]. Hence to face such a changed environment, the animal should certainly undergo changes in its body constitution agreeable to that of the altered environment,[2,3] leading to changes in the physiology of the organisms. Hence, some preliminary investigations were conducted on the fish, *Tilapia mossambica* exposed to sublethal concentrations of methyl parathion in static water media. The parameters investigated include the changes in the whole body weight, water content and sodium, potassium and calcium ion contents. Though simple, these parameters are of profound importance in maintaining the homeostatic balance under methyl parathion imposed stress condition.

Materials and Methods

The details of the maintenance, acclimation, feeding and determination of LC_{50} value of methyl parathion to *T. mossambica* were described earlier.[4,5]. LC_{50} was found to be 0.27 p.p.m. for 48 hr[5]. Hence 0.09 p.p.m. concentration of methyl parath on was selected, since it is a sublethal concentration and the fishes weighing 8.0 ± 2.0 g were sorted into six batches of 10 each and exposed for 48 hr. The troughs containing normal and methyl parathion exposed (MPE) fishes were aerated frequently to prevent hypoxic condition of the medium.

Body weight changes were determined at 12 hr intervals. The water content was determined by drying them in a hot air oven at 80°C for 24 to 48 hr. The process was continued till no changes in weight was observed. The muscle, gill, liver and brain tissues of normal and MPE fishes were wet ashed[6] and sodium, potassium and calcium were estimated using Elico flame photometer. The results were analysed by student 't', test.[7]

Results and Discussion

The body weight of the MPE fish showed a slight decrease throughout the study up to 48 hr (Table 1), which may be either due to the utilisation of organic reserves or due to the loss of body water or may be due to both. A similar loss of body weight was reported in the house sparrows treated with DDT[8], fish[9] and snails[10] under sublethal concentrations of malathion exposure. Since the loss of weight is associated with susceptibility to pesticides[11], the gradual decrease in the body weight suggests the possibility of fatality on prolonged exposure, probably due to the loss of some body constituents. Since the water content showed fluctuations (Table 1), the progressive loss of body weight during methyl parathion exposure may involve the loss of some body constituents other than water. The un-

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TABLE 1. CHANGES IN THE BODY WEIGHT AND WATER CONTENT OF NORMAL AND METHYL PARATHION (MPE) EXPOSED FISH FOR DIFEFRENT HOURS

Constituent	12 hr exposure		24 hr exposure		36 hr exposure		48 hr exposure	
	Normal	MPE	Normal	MPE	Normal	MPE	Normal	MPE
Body weight (g)	10.175 ±1.138	10.095 ±1.129 (−0.8)	10.162 ±1.038	10.008 ±1.096 (−2)	10.152 ±0.988	9.932 ±1.088 (−2)	10.125 ±1.023	9.757 ±1.087 (−4)
Water content (g/g fish)	0.718 ±0.007	0.706 ±0.012 (−2)	0.712 ±0.009	0.704 ±0.008 (−1)	0.714 ±0.010	0.723 ±0.009 (+1)	0.716 ±0.009	0.728 ±0.012 (+2)

Values are mean ± S.D. of 6 individual observations.
None of them were statistically significant from the control.
Figures in parenthesis indicate decrease (−sign) or increase (+sign) over eontrol.

changed water content in the MPE fishes might also suggest the maintenance of osmotic balance during the stress condition. It is likely that this osmotic balance is the result of salt pump rather than water pump from the body to the medium, aimed towards maintaining the structural and functional properties of the cell under methyl parathion exposure.

The Na^+, K^+ and Ca^{++} in the muscle, gill, liver and brain tissues of 48 hr MPE fishes showed a decrease (Table 2). Except for the K^+ and Ca^{++} contents in muscle and brain tissues, the decrease in Na^+, K^+ and Ca^{++} contents in all the four tissues were statistically significant. Eisler and Edmunds[12] reported a decrease in the Na^+, K^+ and Ca^{++} contents of the liver of fish exposed to endrin. The decrease in the ionic content of MPE tissues evidently suggest the change in the permeability properties of different biological membrane systems under methyl parathion exposure. It is reported that under stress conditions osmotically active substances like amino acids and metabolites like pyruvate and lactate may be on the increase[3], perhaps to compensate the loss of inorganic ions. An increase in the tissue amino acid and pyruvate contents were also observed in the same fish species[13], thus corrobarating with above observation. In the present study, the methyl parathion stress might have resulted in the hypertonicity of the animal tissues and MPE fish seem to prefer the salt pump over the water pump to maintain the isoosmotic

TABLE 2. CHANGES IN SODIUM, POTASSIUM AND CALCIUM CONTENTS (mM/G WET WT. TISSUE) IN THE MUSCLE, GILL, LIVER AND BRAIN TISSUES OF NORMAL AND METHYL PARATHION EXPOSED (MPE) (48 HR) FISH

Constituent	Muscle		Gill		Liver		Brain	
	Normal	MPE	Normal	MPE	Normal	MPE	Normal	MPE
Sodium	0.0448 ±0.0034	0.0380 ±0.0029** (15)	0.0729 ±0.0033	0.0657 ±0.0020*** (10)	0.0410 ±0.0023	0.0372 ±0.0026* (9)	0.0673 ±0.0033	0.0611 ±0.0023** (10)
Potassium	0.0662 ±0.0073	0.0612 ±0.0045NS (9)	0.0367 ±0.0028	0.0333 ±0.0017* (9)	0.048 ±0.0061	0.0428 ±0.0051NS (11)	0.0422 ±0.0024	0.0410 ±0.0024NS (3)
Calcium	0.0027 ±0.0005	0.0024 ±0.0004NS (11)	0.0380 ±0.0027	0.0325 ±0.0027** (14)	0.0045 ±0.0004	0.0038 ±0.0003+ (15)	0.0043 ±0.0012	0.0038 ±0.0012NS (13)

Values are mean ± S.D. of 6 individual observations.
*Significant at $P<0.05$; **Significant at $P<0.01$; NS, not significant; +Significant at $P<0.025$; ***Significant at $P<0.005$.
Figures in parenthesis indicate % decrease over control.

balance and this might have resulted in a decrease in the ionic content of MPE tissues, without change in the water content of the whole animal.

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References

1. Mohan Rao, G. J., In *Pesticides*, S. K. Majumder Academy of Pest Control Sciences, India, 1968, (Ed), 229.
2. Fry, F.E.J., In *Fish Physiology*, Hoar and Randall, Vol. VI, Academic Press, London, New York, 1971.
3. Hoar, W. S., *General and Comparative Physiology*, Printice-Hall of India Pvt. Ltd., New Delhi, 1976.
4. Siva Prasada Rao, K. and Ramana Rao, K. V., Effect of sublethal concentration of methyl parathion on selected oxidative enzymes and organic constitutents in the tissues of teleost., *T. mossambica* (Peters) *Curr. Sci.*, 1979, **48**, 526.
5. Siva Prasada Rao, K. and Ramana Rao, K. V., Toxicity of methyl parathion to the fresh water teleost, *T. mossambica* (Peters). *Comp. Physiol. Ecol.*, 1982, 7, (in press).
6. Dall, W., Hypo-osmoregulation in crustacea. *Comp. Biochem. Physiol.*, 1967, **21**, 653.
7. Snedecor, G. W., *Statistical Methods*, Iowa State University press, Ames, Iowa, 1956.
8. Bernard, R. F., 1963, In the *Environmental pollution by pesticides*, (C. A. Edwards (Ed)), Plenum press, London and New York, 1974.
9. Kabeer Ahammad Sahib, I., Jagannatha Rao, K. S. and Ramana Rao, K. V., Effect of malathion exposure on some physical parameters of whole body and on tissue cations of teleost, *T. mossambica* (Peters). *J. Biosci.*, 1981, **3**, 17.
10. Sivaiah, S. and Ramana Rao, K. V., Effect of malathion on histo-gravimetry, histosomatic indices and water content in the snail., *Pila globosa* (Swainson)." *Geobios.*, 1979, **6**, 8.
11. Gish, C. D. and Chura, N. J., Toxicity of DDT to Japanese quail as influenced by body weight, breeding condition and sex. *Toxicol. Appl. Pharmacol.*, 1970, **17**, 740.
12. Eisler, R. and Edmunds, P. H., In *Environmental Pollution by pesticides*, C. A. Edwards (Ed), Plenum press, London and New York, 1974.
13. Siva Prasada Rao, K., Studies on some aspects of metabolic changes with emphasis on carbohydrate utility in the cell-free systems of the teleost, *Tilapia mossambica* (Peters) subjected to methyl parathion exposure. *Ph.D. Thesis*, S. V. University, Tirupati, 1981.

RESEARCH NOTES

OXYTETRACYCLINE FERMENTATION: STRAIN SELECTION OF *STREPTOMYCES RIMOSUS* FOR IMPROVED PRODUCTION OF OXYTETRACYCLINE

Ultraviolet light and X-rays were used to improve yields of oxytetracycline by *Streptomyces rimosus*. A rapid method to isolate improved strain for oxytetracycline production was employed after the preliminary screening of morphological mutants, but it did not prove efficient as the colony which gave a greater zone of inhibition did not always produce a higher antibiotic titre in liquid media. UV-light at a dose level giving about 8% survival resulted in the production of mutants which enhanced antibiotic activity and gave oxytetracycline production 140% better than the original strain of *S. rimosus*, and was superior to other doses of UV-light and X-rays.

Mutations and selection have been used for many years effectively for the isolation of strains of fungi[1,2] and actinomycetes[3] with improved and higher antibiotic production. The technique has been used for the development of new strains with increased production of oxytetracycline[4,5], but without much success. The information is limited and there is always a demand for highly improved strains in the competitive field of industrial production. Many mutagenic agents are used for improved strain selection. In our present study, an attempt has been made to develop high yielding strains of *Streptomyces rimosus*, known to produce oxytetracycline, using UV-light and X-rays as mutagens.

A strain of *S. rimosus*[6] producing oxytetracycline was maintained on tryptone-glucose yeast agar slants. Spore suspension was made with sterile distilled water and the viable spore density was adjusted to 5.5×10^7 counts per ml of the suspension. The mutagenic agents employed in this experiment were ultraviolet light (Philips germicidal tube, 15 Watts, 230-250 mμ wave length) and X-rays (RADON type, 110 KV-and 11.2 mA). One ml of the spore suspension taken in a small petri dish was exposed to UV-light at a distance of 16 cm with frequent shaking. In treatment with X-rays, one ml of spore suspension of an improved strain selected after UV-radiation was taken in carrel flask, and was subjected to exposure with X-rays.

The treated spores were plated on the agar medium and the colonies on the plates were flooded with soft nutrient agar seeded with the test organism, *Bacillus cereus*, to measure the potency[7] of the colonies, which were morphologically different from the parent and selected in the preliminary screening as morphological mutants. The morphological mutants, selected from different stages of treatment, were finally tested for antibiotic acitivity by a shake flask process in the fermentation medium consisting of 1 per cent corn starch, 1 per cent soybean meal, 0.3 per cent $NaNO_3$. 0.1 per cent enzyme hydrolysed casein, 0.02 per cent $CaCO_3$ and 100 ml distilled water (pH 7.0). Each 250 ml Erlenmeyer flask containing 40 ml medium was inoculated with 2.5 per cent (V/V) seed culture of vegetative mycelium and incubated at 28°C for 4 days on a rotary shaker (150 r.p.m.). The antibiotic concentration in fermentation broth was determined by the conventional cup-plate method[8] using *B. cereus* as the test organism. The mycelial growth was measured by taking the weight of dry cells kept at 80 ± 5°C in an oven for 24 hr.

Exposure of spores of *S. rimosus* producing oxytetracycline to ultraviolet light for 60 and 90 min. giving about 8 per cent and 0.04 per cent survival respectively, resulted in the production of morphological mutants among the survivors, of which 20 per cent gave higher yields and 66 per cent exhibited activities of oxytetracycline production. An improved strain obtained from UV-radiation was further treated with 25 and 30 krad of X-rays resulting 98.1 and 99.9 per cent killing respectively. But none of the doses of X-rays was effective in producing superior colony among the morphological mutants from the survivors with the exception of low producers (33 per cent at a dose of 25 kilorads and 66 per cent at 30 kilorads).

The zone of inhibition of the test organism as a measure of the potency of the mutants: The relationship between zone-diameter and the antibiotic potency of some of the morphological mutants is shown in Table 1. It was observed that the colony which gave a greater zone of inhibition did not always give a higher antibiotic titre in liquid media. Some colonies which were overgrown by the sensitive organism gave maximum yield of oxytetracycline in the fermentation broth. The colony size of the isolates was more or less the same and their morphology did not differ markedly from the parent except some pinkish shade, star-like structure, distinct corners with central hole, uneven surface and serrated edges in some of the colonies. Therefore, the zone-diameter in flooding technique cannot be used as an assured means for quick isolation of high yielding mutants of *S. rimosus*.

Effect of ultraviolet and X-rays on cultural characters and antibiotic potency of S. rimosus: Effect of UV-

TABLE 1. RELATIONSHIP BETWEEN ZONE-DIAMETER AND ANTIBIOTIC POTENCY AMONG THE MORPHOLOGICAL MUTANTS AFTER TREATMENT WITH UV-LIGHT AND X-RAYS

Mutants	UV radiation time (min)	X-ray dose (Kr)	Zone-diameter (mm)	Oxytetracycline in broth (μ g/ml)
UV-60/1	60	—	20	37
UV-60/2	60	—	30	37
UV-60/3	60	—	32	77
UV-60/4	60	—	18	103
UV-60/5	60	—	30	28
UV-60/6	60	—	25	43
UV-60/7	60	—	Nil	32
UV-60/8	60	—	24	28
X-25/141	—	25	17	120
X-25/142	—	25	10	105
X-25/143	—	25	12	120
X-25/144	—	25	7	77
X-25/145	—	25	Nil	120
X-25/146	—	25	Nil	120
X-30/141	—	30	15	50
X-30/142	—	30	19	120
X-30/143	—	30	18	110
X-30/144	—	30	28	120
X-30/145	—	30	Nil	38
X-30/146	—	30	13	90

light, and X-ray exposure of *S. rimosus* on cultural characters and antibiotic production is shown in Tables 2 and 3. On treatment with ultraviolet light for 60 and 90 min, more than hundred colonies from those surviving the treatment were selected as morphological mutants, out of which only 18 isolates were listed in Table 2, showing 4 as high-yielding and 9 as low-yielding types. On further treatment of an improved strain UV-60/14 with X-rays, 12 colonies were selected at random from the morphological mutants and were tested for antibiotic potency and cultural changes in liquid media, but none exhibited better activities (Table 3). Treatment with ultraviolet light for 60 min. produced superior mutants, which gave 80-140 per cent better production of oxytetracycline than the original strain of *S. rimosus*. There was no relationship between mycelial growth and oxytetracycline production, but it was noted that all the high-yielding mutants showed more rapid and abundant growth than the parent. Although the colonies of the mutants did not differ so far from the parent, in shake flask, the high-yielding mutants showed variability in cultural characters by granular growth and black pigmentation, and low-producers by flaky mycelium and yellow pigment from the parent strain whose mycelium was of globular type and colour of pigment was brown.

TABLE 2. MUTAGENIC EFFECT ON CULTURAL CHARACTERS AND OXYTETRACYCLINE PRODUCTION BY MORPHOLOGICAL MUTANTS FROM A PARENT STRAIN OF *S. RIMOSUS* EXPOSED TO UV-LIGHT

Mutants	UV radiation time (min)	Mycelial growth (mg/ml)	Morphology in liquid media	Pigmentation (broth colour)	Oxytetracycline in broth (μ g/ml)
Parent	60	6.1	globular	brown	50
UV - 60/4	60	7.6	granular	black	103
UV - 60/5	60	6.2	flaky	yellow	28
UV - 60/11	60	7.4	granular	black	90
UV - 60/14	60	8.3	granular	black	120
UV - 60/18	60	5.4	flaky	yellow	27
UV - 60/20	60	4.8	flaky	yellow	32
UV - 60/24	60	6.7	globular	brown	50
UV - 60/25	60	6.5	flaky	yellow	37
UV - 60/28	60	6.9	granular	black	105
UV - 60/36	60	8.1	flaky	yellow	40
UV - 60/39	60	7.7	granular	black	67
UV - 90/1	90	4.7	flaky	yellow	38
UV - 90/2	90	5.5	flaky	yellow	37
UV - 90/4	90	6.9	globular	brown	58
UV - 90/6	90	6.1	flaky	yellow	32
UV - 90/9	90	7.5	globular	brown	57
UV - 90/15	90	6.1	flaky	yellow	32
UV - 90/18	90	6.2	globular	brown	50

TABLE 3. MUTAGENIC EFFECT ON CULTURAL CHARACTERS AND ANTIBIOTIC PRODUCTION BY MORPHOLOGICAL MUTANTS FROM A MUTANT STRAIN UV – 60/14 EXPOSED TO-X-RAY

Mutants	X-ray dose (Kr)	Mycelial growth (mg/ml)	Morphology in liquid media	Pigmentation (broth colour)	Oxytetracycline in broth (μ g/ml)
UV – 60/14	25	8.3	granular	black	120
X – 25/141	25	8.9	granular	black	120
X – 25/142	25	8.5	granular	black	105
X – 25/143	25	8.3	granular	black	120
X – 25/144	25	6.2	globular	brown	77
X – 25/145	25	7.7	granular	black	120
X – 25/146	25	6.9	granular	black	120
X – 30/141	30	7.9	flaky	yellow	50
X – 30/142	30	8.2	granular	black	120
X – 30/143	30	7.6	granular	black	110
X – 30/144	30	7.8	granular	black	120
X – 30/145	30	6.4	flaky	yellow	38
X – 30/146	30	7.1	granular	black	90

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References

1. Backus, M. P. and Stauffer, J. F., The Production and selection of a family of strains in *Penicillium chrysogenum*. *Mycologia.*, 1955, **47**, 429.
2. Dulaney, E. L., Observation on *Streptomyces griseus*, Further studies on strain selection for improved streptomycin production. *Mycologia.*, 1953, **45**, 481.
3. Alikhanian, S. I. and Borisova, L. N., Recombination in *Actinomyces aureofaciens.*, *J. Gen. Microbiol.*, 1961, **26**, 19.
4. Alikhanian, S. I., Mindlin, S. Z., Goldat, S. U. and Vladimizov A. V., Some regularities in induced variation and selection of tetracycline—producing actinomycetes., *Ann. N. Y. Acad. Sci.*, 1959, **81**, 915.
5. Alikhanian, S. I., Induced mutagenesis in the selection of microorganism. *Adv. Appl. Microbiol.*, 1962, **4**, 1.
6. Mandal, S. K., Mukhopadhyay, D. and Roy, D. K., Fermentative production of antibiotic by actinomycetes: Screening of organisms and production of oxytetracycline by *Streptomyces rimosus.*, *Indian J. Microbiol.*, 1981, **21**, 28.
7. Banerjee, A. B. and Bose, S. K., A rapid method for isolating mutants of *Bacillus subtilis* producing increased or decreased amounts of the antibiotic., Mycobacillin. *J. Appl. Bact.*, 1964, **27**, 93.
8. Grove, D. C. and Randall, W. A., *Assay Methods of Antibiotics—A Laboratory Manual.*, Medical Encyclopedia, N. Y., 1955, 50.

PHYSICO-CHEMICAL CHARACTERISTICS AND FATTY ACID COMPOSITION OF SOME IMPORTED AND INDIGENOUS VARIETIES OF RAPESEED OIL

Rapeseed (*Brassica campestris*) oil extracted from eight imported cultivars had erucic acid content ranging from 0 to 3.8 per cent in contrast to 48.8 to 55.4 per cent in indigenous varieties. Distinct differences in Iodine value, saponification value and Bellier turbidity temperature value were observed between imported and indigenous rapeseed oils. Indigenous rapeseed varieties had 7.8 to 13.6 per cent oleic acid, whereas imported varieties had 55.8 to 64.9 per cent. Poly unsaturated fatty acids were present to the extent of 36 per cent in imported oils in contrast to 27 per cent in indigenous rapeseed oil.

Rapeseed (*Brassica campestris*) oil differs from other vegetable oils as they contain 45-55 per cent of erucic acid (cis. 13-docosenoic acid)[1]. The use of high erucic acid oil in the diet may cause myocardial fibrosis[2]. New varieties of rapeseed, low in erucic acid, have been developed in Canada and Sweden.

The results of the analysis on the extracted oil from the imported and indigenous rapeseeds are presented in this communication.

Six rapeseed and one oil sample from Canada (var. 'Candle', 'Tower', 'Canola-3', Alberta-2', 'Canola' (*B. campestris* + *B. napus*), 'Saskat chewan', and refined Canola' oil), one refined rapeseed oil from Sweden, one rapeseed (var. 'T-59') and one mustard seed (var.'RT-11',

from Agricultural Research Station, Haryana (India), and one *mustard sample* from Mysore market, were used. The seeds were cleaned by sieving and hand picking. About 50 g seeds in each case was powdered in a waring blender. The oil was completely extracted using petroleum ether (40-60°C) solvent in a soxhlet apparatus. Solvent was removed under reduced pressure in a rotary evaporator. The filtered oil was used for the analysis. Butyro-refractometer reading (BRR) of the oil samples was determined at 40°C using an oil refractometer (Advance Research Instruments Co., New Delhi). Bellier turbidity temperature (acetic acid method). iodine value (Wij's), saponification value and free fatty acid contents were determined according to AOCS procedures[3].

The sodium methoxide catalysed transesterification

TABLE 1. PHYSICAL AND CHEMICAL CHARACTERISTICS OF IMPORTED AND INDIGENOUS RAPESEED OILS AND MUSTARD SEED OIL

Variety	Butyro-refracto-meter reading at 40°C	Refractive index	Bellier turbi-dity temp. (°C)	Iodine value (Wijs')	Saponification value	Unsaponifaible matter (%)	F.F.A (as oleic acid) (%)
Candle	61.0	1.4667	15.5	121.3	187.3	1.50	0.96
Tower	60.0	1.4659	16.0	118.2	191.2	1.42	0.86
Canola-3	60.0	1.4659	17.0	121.2	182.8	1.40	1.54
Alberta-2	60.0	1.4659	16.5	121.5	190.0	1.45	1.40
Canola	60.0	1.4659	17.08	121.4	189.9	1.40	0.70
Saskatchewan	58.0	1.4646	17.0	115.7	196.0	1.42	0.45
Canola oil (refined)	60.0	1.4659	16.5	121.0	190.0	1.20	0.20
Rapeseed oil (Sweden)	60.0	1.4659	17.0	120.0	189.5	1.30	0.20
T-59 (Haryana)	58.0	1.4646	28.3	106.0	170.2	1.00	0.90
RT-11 (Haryana)*	58.5	1.4636	27.1	106.0	170.5	0.97	0.82
Mysore sample*	59.0	1.4652	28.5	107.0	171.4	0.97	0.98

*Mustard oil samples

TABLE 2. FATTY ACID COMPOSITION OF RAPESEED OIL EXTRACTED FROM IMPORTED AND INDIGENOUS VARIETIES AND MUSTARD SEED OIL

variety	Fatty acid (%) by wt											Iodine value	
	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	Experi-mental	Theore-tical
Candle	T	3.27	0.35	1.20	55.84	22.83	12.04	0.44	1.35	T	2.50	121.3	121.8
Tower	T	3.80	0.40	1.21	64.88	16.30	12.07	0.25	1.18	T	Nil	118.2	117.1
Sample-3	T	4.05	T	0.77	53.73	24.56	11.82	0.21	2.48	T	2.36	121.2	123.6
Alberta-2	T	3.55	T	1.01	58.87	21.12	13.53	0.20	1.83	T	1.89	121.5	123.9
Canola	T	3.59	0.46	0.81	53.94	24.92	11.95	0.36	2.03	T	1.84	121.4	124.5
Saskatchewan	T	4.40	0.35	0.64	59.43	23.03	9.55	0.44	1.46	T	0.60	115.7	118.1
Rapeseed oil (Sweden)	T	5.90	T	1.80	58.90	20.20	13.20	T	T	T	Nil	121.0	120.3
Canola oil (refined)	T	5.80	T	1.30	61.20	14.20	13.20	T	0.50	T	3.80	120.0	115.5
T-59 (Haryana)	Nil	2.90	T	1.80	10.10	14.10	10.70	0.50	4.00	0.42	55.40	106.0	106.7
RT-11 (Haryana)*	Nil	2.40	T	0.70	7.80	15.60	10.20	0.56	4.90	0.46	55.40	106.0	107.0
Mysore samples*	Nil	2.80	T	1.10	13.60	16.50	10.50	0.60	5.70	0.40	48.80	107.0	107.6

T = Traces, ←—— <0.3
*Mustard oil samples.

procedure[4] was followed for the preparation of fatty acid methyl esters. Separation of the esters was carried out using a CIC dual column chromatograph equipped with hydrogen flame ionisation detector, under the following conditions: Stainless steel column (5′ × 1/8″) packed with 15 per cent diethylene glycol succinate on Chromosorb W (80-100 mesh); column temperature 185°C isothermal; injector and detector ports adjusted to 240°C; carrier gas, N_2 15 ml/min; H_2, 20 ml/min. Identification of the fatty acids was made by comparison with standards (Sigma). Quantitation of the peaks was done by triangulation. Each analysis was carried out in triplicate and the average is reported.

The results presented in Table 1 show that iodine value of the imported rapeseed oils is higher (115.7 to 121.5) than the oils from indigenous varieties of rapeseed and mustard oils (106 to 107). This is reflected in the fatty acid composition presented in Table 2. Bellier turbidity temperature value ranged between 15.5 and 17.0°C for imported rapeseed oils, whereas it was between 27.1°C and 28.5°C for indigenous rapeseed and mustard oils. Unsaponifiable matter was present upto 1.5 per cent in the imported rapeseed oils, in contrast to 1.0 per cent in indigenous rapeseed oils. In general, as can be seen from Table 2, low erucic rapeseed oils contained higher oleic, linoleic and linolenic acids. This is in accordance with earlier observation[1]. The experimentally determined iodine values were in agreement with the calculated values (Table 2). The lower Bellier turbidity temperature value (below 17°C) obtained for imported rapeseed oils may be attributed to the presence of lower amounts of arachidic and behenic acids than in indigenous oils. Linolenic acid content in imported rapeseed oils ranged between 12 and 13.5 per cent, whereas it was 10.2 to 10.7 in indigenous oils.

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References

1. Appelquist, L. A. and Ohlsson, R., in *Rapeseed: Cultivation, Composition, Processing* and *Utilisatiom*, Elsevier Co., Amsterdam, The Netherlands, 1972, 33.
2. Beare-Rogers, J. I., Nutritional aspects of long chain fatty acids, *Proc. International Conference on Science, Technology and Marketing of Rapeseed and Rapeseed Products*, Rapeseed Association of Canada, 1970, 450.
3. *Official and Tentative Methods*, American Oil Chemists Society, U.S.A., 3rd Edn., 1973.
4. Christie, W. W., *Lipid Analysis*, Pergamon Press, New York, 1973.

A SIMPLE DROPPING METHOD FOR DETERMINING THE TEXTURE IN PARBOILED RICES

Optimally cooked, mild to severely parboiled rice samples after 1 hr of cooking are taken in a rectangular box having flap doors at the bottom. On instantaneously opening the flap doors, the grains fall on a glass plate kept 75 cm below the box. The glass plate is marked with concentric circles of 5 cm radial difference and the grains that occupy different circles are collected separately and weighed quickly. More severe the parboiling, greater was the area to which the cooked rice got distributed. Based on the proportion of grains that occupied the first two circles, the parboiled samples could be graded for their texture. Samples were graded as 'tough', when more than half of the grains got distributed beyond 10 cm radius from the central point of drop.

In recent years, there is a spurt in parboiled rice production in the traditionally raw rice milling areas like Punjab and Andhra Pradesh resulting in flooding of the market with different types of parboiled rices. The existing specifications[1] for parboiled rice do not stipulate any criteria for accepting rice based on its palatability characteristics and consequently the traders face with certain problems in disposing of rice in consuming areas like Kerala. Though certain instrumental tests[2-5] for determining the texture of cooked rice have been developed and some[6,7] have been used for assessing the texture of parboiled rice, the need for developing a simple test was felt. Hence, the following dropping method is proposed.

Six month old 'IR 20' paddy was soaked in cold (CS)[8], warm (WS)[9] and hot (HS)[10] water and steamed at 0 psig for 10 min. One lot of 'IR 20'—WS was steamed at 0 psig for 20, 30 and 40 min also. WS samples of 'TKM 9' and 'Co 25' were parboiled at 80, 90, 100, 110 and 120°C for 10 min[11]. One lot of paddy was soaked in water at room temperature for 30 min, steamed at 0 psig for 10 min followed by 5 psig for 20 min and 15 psig for 30 min (designated as PP). All these samples were shade dried, dehulled in a Satake grain testing mill and polished in a McGill miller No. 3 to 6±0.1 per cent degree of milling.

Rice was cooked in excess water to its optimal cooking time[12]. As the optimal cooking time could not be

determined in 110°C, 120°C, and PP samples by the customary glass-plate opaque-core method, these samples were cooked for 2 min more than the 40-min steamed samples[13]. After the completion of cooking, the excess cook water was drained and the sample transferred to petridish containing a filter paper inside the lid. The petridish was placed in a water saturated chamber and the samples tested after 1 hr of cooking. The equilibrated moisture content of rice upon soaking at room temperature (EMC-S) was also determined[14].

Device for dropping the cooked rice: Fifteen grams cooked rice kept uniformly distributed in a rectangular sample box (A) of 4×4×4 cm made of acrylic sheet was momentarily dropped over a glass plate (B) of 60×60 cm placed on the floor by releasing the bottom flaps (C) by turning the release lever (D) which supports the flaps (Fig. 1). Concentric circles of 5 cm radial distance are marked on the glass plate from the central point of drop. The cooked grains were dropped from a height of 50 and 75 cm on the glass plate and the grains that occupied each circle were quickly collected and weighed separately. The test was repeated thrice.

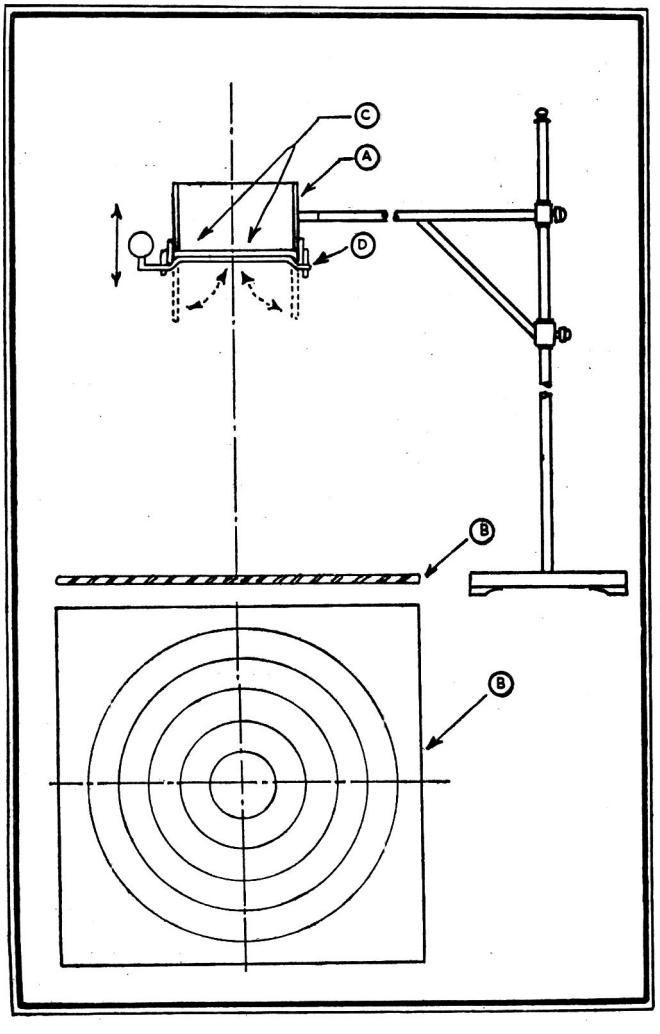

Fig. 1. Schematic diagram of dropping device

A. Sample holder, B. Glass plate, C. Bottom flaps, D. Release

TABLE 1. DISTRIBUTION OF COOKED PARBOILED RICE DROPPED FROM 75 CM HEIGHT (%)

Treatment	I circle	II circle	I+II circle
10 min. steamed	61.4	31.3	92.7
20 ,, ,,	51.3	28.4	79.7
30 ,, ,,	37.6	21.3	58.9
40 ,, ,,	24.7	12.3	37.0
Cold soaked	60.0	25.9	85.9
Warm soaked	55.1	22.8	77.9
Hot soaked	25.3	29.0	54.3
Pressure parboiled	19.3	15.1	34.4

Wide distribution of samples on the glass plate occurred while dropping from a height of 75 cm and hence the results pertaining to 75 cm height alone were considered. The quantity of grains that remained within 10 cm radius from dropping point was considerably low in case of severely parboiled samples (Table 1) indicating their tough textures. In case of 40-min steamed and PP samples, the grains occupying the first circle were less than 25 per cent of the samples taken up for the test, whereas,

TABLE 2. COOKED RICE FALLING WITHIN THE FIRST TWO CIRCLES AND THEIR EMC-S VALUES

Parboiling temp. (°C)	Variety	% grains within the Ist two circles	EMC-S (%, d.b.) of polished rice
Raw	Co 25	91.7	41.9
	TKM 9	80.4	39.6
80	Co 25	78.3	75.8
	TKM 9	66.0	68.9
90	Co 25	68.9	107.7
	TKM 9	65.2	83.9
100	Co 25	59.1	121.6
	TKM 9	56.5	94.1
110	Co 25	44.4	139.6
	TKM 9	50.1	109.9
120	Co 25	41.3	145.4
	TKM 9	45.7	124.8
PP	Co 25	28.9	167.8
	TKM 9	44.2	136.1

PP: Pressure parboiling

they were more than 50 per cenr in 10- and 20-min steamed and CS and WS samples. Except in 40-min steamed 110, 120°C and PP samples, in all other cases the grains that occupied the first two circles formed more than 50 per cent of the samples taken up for the test. Negative correlation ($r = -0.936^{**}$) was observed between the proportion of grains that occupied the first two circles and the EMC-S values (Table 2). The quality of the cooked rices parboiled for a longer time or at temperatures above 100°C was poor[15]. This test also indicates a similar pattern in quality. While testing optimally cooked raw samples, appreciable quantity stuck to the sides and the flap doors of the box. Because of the simplicity and rapidity of this test, this method would be useful in grading parboiled rice texture at field level.

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References

1. *Uniform Specifications for Paddy and Rice*, Ministry of Agri. and Irri. Government of India, New Delhi, 1979.
2. Hampel, G. Research on the Quality of White Rice. *Getr. Mehl.*, 1965, **11**, 132.
3. Manohar Kumar. B., Upadhyay, J. K. and Bhattacharya, K. R., Objective tests for the stickiness of cooked rice. *J. Texture Studies.*. 1976 **7**, 271.
4. Bhattacharya, K. R., Sowbhagya, C. M. and Indudhara Swamy, Y. M., Importance of insoluble amylose as a determinant of rice quality. *J. Sci. Fd Agric.*, 1978, **29**, 359.
5. Blakeney, A. B., Instron measurement of cooked-rice texture, *in Proc. Workshop on Chemical Aspects of Rice Grain Quality*, International Rice Research Institute, 1979, 343.
6. Mohandoss, R. and Pillaiyar, P., An extrusion test for determining the palatability of parboiled rices. *J. Fd Sci.Technol.*, 1980, **17**, 244
7. Pillaiyar, P. and Mohandoss, R., A Pressing-device to measure the texture of cooked rice. *J.Texture Studies.*, 1981, **12** 473.
8. Pillaiyar, P., Commercial parboiling methods. *Kisan Wld.*, 1980, 7, 32.
9. Bhattacharya, K. R. and Indudhara Swamy, Y. M., Conditions of drying parboiled paddy for optimum milling quality. *Cereal Chem.*, 1967. **41**, 592.
10. *Parboiling of Paddy*, Circular No. 7 (revised), Central Food Technological Research Institute, Mysore, 1960.
11. Pillaiyar, P and Mohandoss, R., Hardness and colour in parboiled rices produced at low and high temperatures. *J. Fd Sci. Technol.*, 1981, **18**, 7.
12. Bhattacharya, K. R. and Sowbhagya, C. M., Water uptake by rice during cooking. *Cereal Sci. Today.*, 1971, **16**, 420.
13. Pillaiyar, P. and Mohandoss, R., On the Completion of cooking in rice. *Indian J. Nutr. Dietet.*, 1981, **18**, 385.
14. Indudhara Swamy, Y. M., Ali, S. Z. and Bhattacharya, K. R., Hydration of raw and parboiled rice and paddy at room temperature. *J. Fd Sci. Technol.*, 1971, **8**, 20.
15. Pillaiyar, P. and Mohandoss, R., Cooking qualities of parboiled rices produced at low and high temperatures. *J. Sci. Fd Agric.*, 1981, **32**, 475.

EFFECT OF HEAT TREATMENTS ON STABILITY OF ASCORBIC ACID IN COPPER CONTAMINATED MILK OF COWS AND BUFFALOES

Losses in ascorbic acid content during heating and storage of cow and buffalo milk contaminated with copper are reported. Compared to cow milk, the losses were significantly less in buffalow milk. During storage for 48 hr at 5 ± 1°C, ascorbic acid was almost completely destroyed in cow milk contaminated with 0.4 ppm copper; the corresponding losses in buffalow milk were 25-40%.

Ascorbic acid is very sensitive to light, heat and copper. Pasteurization destroys appreciable quantities of ascorbic acid in cow milk, especially when it is contaminated with copper[1-3]. Earlier work[4] has shown that in buffalo milk, copper is less detrimental to the stability of ascorbic acid. Losses of ascorbic acid during heating and storage of buffalo milk contaminated with copper are reported here. For the purpose of comparison, data on cow milk are presented.

Milk from cows and buffaloes was collected individually from four animals each, directly in brown glass bottles. The milk was pooled in another brown bottle and divided into two portions. Copper sulphate solution was added to one portion to give it an added copper content of 0.4 ppm. Samples with and without added copper were further divided into four parts and subjected to four different types of heat treatments. These treatments were (*i*) without heating, the samples were kept at room temperature, (*ii*) heating at 63°C for 30 min in a thermostatic water bath (L.T.L.T. treatment) (*iii*) heating at 71°C for 15-20 sec (H.T.S.T. treatment), and (*iv*) heating just to boil, followed by immediately cooling to room temperature (27°C) with chilled water. Ascorbic acid was estimated immediately and after a storage period of 48 hr (in a refrigerator) by the method of Pelletier[5].

The average ascorbic acid content of fresh cow milk was 17.1 mg/l and that of buffalo milk was 22.8 mg/l. Heating reduced ascorbic acid content in cow and buffalo milk as shown in Table 1. The loss was 10.6, 4.7 and 6.8 per cent during LTLT, HTST and boiling, respectively in cow milk. In buffalo milk, the corresponding values were 7.4, 4.2 and 5. 4 per cent respectively.

TABLE 1. ASCORBIC ACID CONTENT (M/G1) OF COPPER CONTAMINATED RAW AND HEATED MILK

Treatment	Cow milk		Buffalo milk	
	Uncontaminated	Contaminated*	Uncontaminated	Contaminated
Raw milk	17.1	14.8	22.8	20.5
L.T.L.T.	15.3	11.3	21.2	18.5
H.T.S.T.	16.3	13.5	21.9	20.2
Boiling	15.9	13.7	21.6	19.3

*Level of copper contamination 0.4 ppm

Presence of copper influenced the degree of loss. With 0.4 ppm copper, the milk samples lost more than 10 per cent of its ascorbic acid content in about 2 hr. After LTLT, HTST and boiling, the losses in cow milk were 34.0, 21.1 and 20.2 per cent and in buffalo milk 18.9, 11.6 and 15.7 per cent respectively. The destruction of ascorbic acid during heating of buffalo milk was less in the presence of copper.

It is reported that pasteurization of milk by LTLT generally results in about 20 per cent decrease of ascorbic acid, while the loss by HTST method is much less. In the present study, even though the destruction of ascorbic acid was less by HTST than LTLT treatment, this difference was not significant either in cow milk or in buffalo milk which are free from copper contamination. In copper contaminated cow milk however, LTLT treatment caused significantly higher destruction of ascorbic acid than the other two heat treatments. Unlike in cow milk, LTLT treatment—compared to the other two treatments-did not destroy significantly higher quantity of ascorbic acid in buffalo milk.

Ascorbic acid content of stored milk as influenced by different heat treatments is presented in Table 2. Considerable destruction of ascorbic acid occurred during storage of milk depending upon the treatments the samples received. In cow milk samples free from copper contamination, about 25 per cent of the ascorbic acid was destroyed during refrigerated storage for 48 hr, irrespective of the initial heat treatments given to the sample. In copper contaminated milk, the destruction of ascorbic acid was almost complete during this period except in boiled samples. The losses in boiled milk were comparatively less, probably due to the sulphydryl group activated during boiling[6-7], which acts as an antoxidant[8]. The losses of ascorbic acid during storage was noticeably less in buffalo milk compared to cow milk, particularly in samples contaminated with copper to the same degree. Ascorbic acid was almost completely destroyed in copper contaminated cow milk during storage for 48 hr, about 25-40 per cent losses were noticed in buffalo milk. This marked difference in the stability of ascorbic acid could be due to differences in the association of copper with the constituents of milk. It has been reported that complexing of copper alters its catalytic activity[9]. Therefore, changes in the association pattern of copper in milk would affect its influences on ascorbic acid.

TABLE 2. ASCORBIC ACID CONTENT (/MG/1) OF COPPER CONTAMINATED RAW AND HEATED MILK* STORED FOR 48 HR AT $5 \pm 1^{\circ}C$

Treatment	Cow milk		Buffalo milk	
	Uncontaminated	Contaminated	Uncontaminated	Contaminated
Raw milk	13.2	0.9	21.2	15.1
L.T.L.T.	11.9	0.7	18.7	14.1
H.T.S.T.	12.6	1.0	19.7	17.1
Boiling	12.9	9.3	19.1	16.2

*Ascorbic acid contents of fresh cow and buffalo milk were 17.1 and 22.8 mg/1 respectively.

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References

1. Gjessing, E. C. and Trout, G. M., Ascorbic acid and oxidized flavour in milk. II. The effect of various heat treatments of milk upon the stability of ascorbic acid and the development of the oxidized flavour, 1940, **23**, 373.
2. Stribley, R. C., Nelson, Jr., C. M., Robert, E. C. an Bermhart, F. W., The effect and interrelationship of copper iron and pasteurizing temperature on the stability of ascorbic acid added to skim milk. *J. Dairy Sci.*, 1950, **33**, 573.
3. Ravikiran, Amma, M. K. P. and Sareen, K. N., Milk fortification with a system containing both iron and ascorbic acid. *Indian J. Nutr. Dietet*, 1977, **14**, 260.
4. Unnikrishnan, V., Sethu Rao, D. and Bhimasena Rao, M., Effect of copper on ascorbic acid content, redox potential and development of oxidized flavour in milk of cows and buffaloes. *J. Milk Fd Technol.*, 1976, **39**, 397.
5. Pelletier, O., Determination of ascorbic acid in enriched evaporated milk. *J. Ass. off. agric. Chem.*, 1967, **50**, 817.
6. Webb, B. H. Johnson, A. H. and Alford, J. A., *Fundamentals of Dairy Chemistry*, 2nd Ed. The AVI Publishing Co., Inc., Westport, Connecticut, 1974.
7. Harland, H. A. and Ashworth, U. S., The preparation and effect of heat treatment on the whey proteins of milk. *J. Dairy., Sci.*, 1945, **28**, 879.
8. Josephson, D. V. and Doan, F. J., Observations on cooked flavour in milk, its source and significance. *Milk Dealer*, 1939, **35**, 29.
9. Monder, C., Williams, J. N. Jr. and Waisman, H. A., The non-enzymatic conversion of dopa to melanin. II. The aerobic catalysis of dopa oxidation by copper ions and copper plasma protein complexes. *Arch. Biochem. Biophys.*, 1957, **72**, 271.

ABSORPTION AND RETENTION OF SULPHUR-DIOXIDE IN RAW MANGO SLICES DURING DRYING AND DEHYDRATION

Raw mango slices after blanching were sulphited by steeping the slices in 1.0, 2.0, 3.0 and 4.0 per cent potasaium meta-bisulphite (KMS) solution for 15 and 30 min. Absorption and retention of SO_2 increased with increasing concentration of KMS solution and duration of steeping. Longer steeping time (30 min) helped better retention of SO_2.

The presence of sulphur dioxide (SO_2) in the dried fruit is essential to preserve natural colour, flavour, and palatability and to prevent nutritional losses, like ascorbic acid, during drying and storage. It is necessary to standardize conditions, whereby enough SO_2 is taken up by the fruit for proper retention after drying. Sulphur dioxide treatment is done either by burning raw sulphur in the presence of fruits or by dipping the fruits in sulphite, bisulphite, or metabisulphite solutions. The absorption and retention of SO_2 depend on variety, general condition and type of fruit[1,2]. In case of pineapple, Bhatia *et al*[3]. observed that steeping in 0.25 to 2.5 per cent potassium metabisulphite solution for 30 min was insufficient to give the desired concentration of SO_2. McBean *et al*[2]. observed that SO_2 concentration in the atmosphere surrounding the fruit was one of the most important factors influencing the uptake of SO_2 and the absorption of SO_2 increased with increase in time.

To assess the possibilities of utilizing dried raw mango slices for preparation of mango chutney, absorption and retention of SO_2 during drying and dehydration of raw mango slices have been studied.

Raw mangoes of required maturity, size and shape were collected from market, peeled, cut into slices, blanched in boiling water for 2.5 min. for peroxidase inactivation[4], and then sulphited. Sulphitation was done by steeping the slices in 1.0, 2.0, 3.0 and 4.0 per cent KMS solution for 15 and 30 min. The ratio of weight of potassium metabisulphite solution to slices was 2:1. Sulphited slices were dried in sun and in cabinet drier at 57°±5°C temperature to 6 per cent moisture level. Absorption of SO_2 during sulphitation and its retention during drying was determined by the method of A.O.-A.C.[5].

The amount of the SO_2 absorbed and retained was directly proportional to the concentration of KMS solution and the time of steeping (Fig. 1 and Table 1).

TABLE 1. ABSORPTION AND RETENTION OF SULPHURDIOXIDE BY RAW MANGO SLICES AS AFFECTED BY CONCENTRATION OF KMS AND TIME OF STEEPING

Method of drying	KMS concn (%)	Absorption of SO_2 (ppm) at indicated periods of steeping		Retention of SO_2 (ppm) at indicated periods of steeping	
		15 min	30 min	15 min	30 min
Sun-drying	1.0	1000 (8333.30)	1248 ((10400)	8800 (93.61)	256 (269.47)
	2.0	1231 (10258.33)	2112 (17600)	500 (544.68)	950 (1000.00)
	3.0	2258 (18866.66)	3480 (29000)	1504 (1600.00)	2288 (24.08.42)
	4.0	3027 (25225.00)	4664 (38866.66)	2421 (2575.53)	5472 (5760.00)
Cabinet drying	1.0	1000 (8333.30)	1248 (10400)	288.00 (303.15)	1056.00 (1111.57)
	2.00	1231 (10258.33)	2112 (17600)	1440 (1515.78)	2816 (2964.21)
	3.0	2258 (18866.66)	3480 (29000)	2764 (2909.47)	5488 (5776.84)
	4.0	3027 (25225.00)	4664 (38866.66)	2816 (2964.21)	5500 (5789.47)

Figures in parentheses indicate values on moisture free basis.

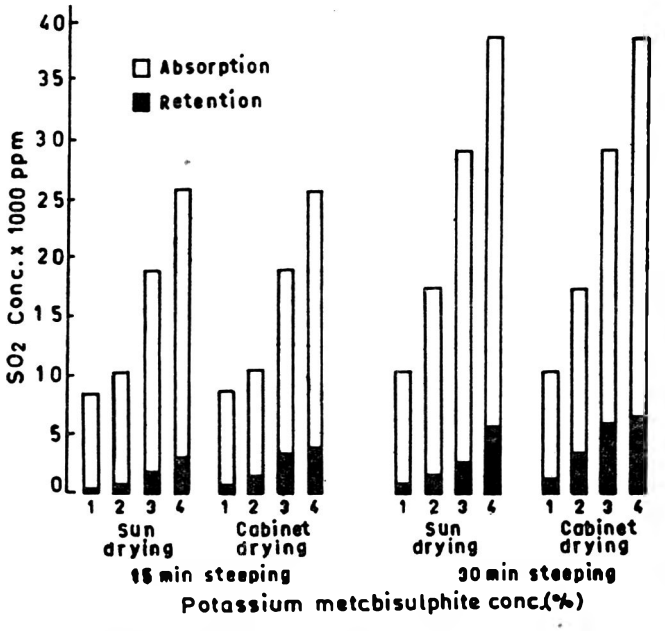

FIG. 1. Relative absorption and retention of Sulphur dioxide in mango slices.

Similar observations were made by McBean *et al*[2]. in apricot, peaches and pears, Long *et al*[1]. and Kikon[6] in apples and Hirway[7] in guava. However, in cabinet drying, above 3 per cent concentration of KMS a corresponding increase in concentration of KMS did not help to have proportional retent on of SO_2. Absorption and retention of SO_2 in all the concentration of KMS was greater in slices steeped for 30 min than for 15 min. Between the two methods of drying, the retention of SO_2 was more in cabinet dried slices as compared to sun-dried samples, irrespective of steeping time and concentration of KMS (Fig. 1 and Table 1).

In the present study, the maximum retention of SO_2 was in those treated with 4.0 per cent KMS for 30 min. This retention was above the upper limit prescribed by F.P.O.[8]. Therefore, to get the maximum retention of SO_2 of 2000 ppm in raw mango slices, sulphitation should be done for 30 min in less than 3 per cent KMS concentration when it is sun-dried, and less than 2 per cent KMS for cabinet drying. However, with 15 min steeping the concentration of KMS should be increased further.

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References

1. Long, J. D., Mark, E. M. and Fisher, C. D., Investigation on the sulphuring of cut fruits for drying. *Calif. Agric. Expt. Sta. Bull.*, 1940, **636**, 1.
2. McBean, D. M., Johnson, A. A. and Pitt, J. I., The absorption of SO_2 by fruit tissue. *J. Fd Sci.*, 1964, **29**, 257.
3. Bhatia, B. S., Amin, H. D. and Lal, G., Studies on dehydration of some tropical fruits. I. Absorption and retention of sulphur dioxide during sulphuring and sulphiting. *Fd Sci.*, 1962, **11**, 76.
4. Khedkar, D. M. and Roy, S. K., Proceedings of the 66th Session, Indian Science Congress, 1979.
5. *Official Methods of Analysis*, Association of Official Analytical Chemists. 11th Ed, 1970, Washington D. C.-20044.
6. Kikon, Y. Y., *Studies on dehydration of apples*. M.Sc. Thesis, I.A.R.I., New Delhi, 1975.
7. Hirway, S. C., *Some studies on drying of guavas*, M.Sc., Thesis, I.A.R.I., New Delhi. 1965.
8. *Fruit Products Order* 1955, Deptt. of Food, Ministry of Agriculture and Irrigation. Govt. of India. 1974.

BOOK REVIEWS

Developments in Food Preservation 1: Edited by Stuart Thorne., Allied Science Publishers Limited, Ripple Road, Barking Essex, United Kingdom, 1981: pp. 272 Price: £ 30.00.

The book comprises of eight chapters on different aspects on the scope and applications of various methods of food preservation.

First chapter deals with the appropriate technology in food preservation for the developing countries. Reasons for very slow progress of food processing industries in the third world countries have been brought out with concrete suggestions for its improvements like, orienting food processing towards national, rather than western food technology; giving more emphasis on drying and pickling; use of solar energy and bio-mass, as energy sources and utilizing plastic pouches as containers.

Second chapter is on cooling aspects of the horticultural produce. Interactions between heat generation during respiration and cooling effect of transpiration have been discussed. Various factors affecting the cooling process, viz., physiological stage of the product. skin thickness, porosity of packing material, vents in the package, air velocity, air temperature etc. are discussed. More dependence on adiabatic temperature rise, rather than carbon dioxide measurement, while calculating heat generation by hortciultural produce has been emphasised.

Latest methods employed for fruit juice concentration are dealt in the third chapter. Due emphasis has been given to exploit the 'Reverse-osmosis' process for production of semi-concentrates from fruit juices due to its inherent advantages. Due to loss of low molecular weight, water soluble aroma components during concentration decrease by this process; author has cautioned to restrict its use to fruits with high acidity and less aroma.

The advantages as well as limitations of microwave processing of foods is dealt at length in chapter four. Usefulness of this heating technique for enzyme inactivation, vegetable dehydration, fruit juice pasteurization and defrosting of frozen products along with its limitations like uneven distribution of the energy inside the cooker and non adjustable nature of the cooker to meet the energy requirements of individual produce have been discussed. Author is of the opinion that though at present, complexity of many foods and biological variations do not assist the application of microwaves, standardization of the cookers followed by the standardization of cooking procedures in a scientific manner would enhance its consumer acceptance.

Chapter five deals with the principle and practices of freeze drying of foods, the freeze drying equipments as well as the economic aspects of this method of preservation. Merits of freeze dried products and need for the proper packaging and storage of such products have been emphasised.

Chapter six deals with 'Extrusion processing'. This most complex system has been presented in a very simple way for proper understanding. The biopolymer aggregate changes are discussed and effect of common ingredients like salt and sugar has been explained using heuristic and hypothetical thearies. Different types of extruders used by the food industry are discussed and the difference in their functional performances are brought out.

The editor himself is the author of the seventh chapter dealing with the effect of temperature on the deterioration of stored agricultural produce. The reactions and the processes responsible for the quality changes during storage of fruits, vegetables and other farm produce have been brought out well in this chapter. The most valuable information furnished by the author is the effect of fluctuating temperatures on the storage changes and the reasons for the differential behaviour of different commodities during storage.

The last chapter is devoted to thermal sterilization of foods. Microbial and enzymatic responses to this method of preservation are explained giving specific examples. Author has brought out the advantage of using stork continuous sterilizer (named as Hydromatic sterilizer) for thermal sterilization of foods. Drastic reduction in the consumption of both steam and water due to deployment of regenearion process is the main advantage of this process, and steam saving upto 60-70 per cent is possible in this case.

This book is very informative, comprehensive and upto-date. Its get up is very good with 37 tables and 99 illustrations evenly distributed and properly presented making the text interesting. The brief summaries of the chapters give adequate idea regarding contents and utility of the text to the reader. A good bibliography comprising of 358 references on the subjects of vital importance in the field of food preservation will be very useful to not only food scientists and technologists, but also to the food processors.

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Food Industry Wastes: Disposal and Recovery: Edited by A. Herzka and R. G. Booth. (Ed) Applied Science Publishers, London & New Jersey, 1981, pp. 246; Prize £ 20.00.

This book is the outcome of a symposium organised by the Association of Consulting Scientists, held in Norwich, U.K. from 11 to 13 Nov. 1980. It contains 17 papers on different aspects of waste disposal and recovery from various food Industries. The first three-quarters of the book deals with methods for separation and disposal of waste particularly solids from food-bearing effluents and the destruction of the solubles in suspension or solution by aerobic or anerobic methods. The latter part deals with processes for recovery of useful food or feed materials from effluents and the feasibility of such processes financially. In the first three papers of the book, the situation relating to legislation and charges for disposal to sewers are considered.

Useful practical details are given for the treatment of specific wastes from dairies, breweries and distilleries, meat and animal by-products, by processes ranging from dissolved air floatation to protein recovery and aerobic treatment of the final irreducible minimum of wastes.

Problems connected with high BOD (Biological Oxygen Demand) food effluents have received detailed attention in recent years, mainly due to increasing awareness of pollution effects, energy price trends and the potential of resource recovery. The two major modern methods of recovery of souble proteins, namely. ultrafiltration and adsorption have been described with sufficient details of membranes and membrane support systems in the case of ultrafiltration and the various adsorbants and methods of using them with concomittant advantages and disadvantages in the latter case. The two methods have been regarded as complimentary rather than competitive. One paper on, recovery of fruit and vegetable waste, describes the possibility of converting the waste into high grade food-stufff biomass with 20-30 per cent protein or converting it into a fuel suitable for many uses.

The authors of the papers are mostly consultants with specialised knowledge in different food industries and have made realistic assessments of the problems associated with effluents. This is a very useful book on a subject of growing importance.

M. KANTHARAJ URS
C.F.T.R.I. MYSORE

Developments in Food Carbohydrate 3.: Edited by C. K. Lee and M. G. Lindley, Applied Science Publishers, London, 1982; pp. Xii+217. Price, £ 26.00.

This book records a comprehensive review on disaccharidases, the carbohydrate degrading enzymes. The articles, clearly written by several outstanding authors, are grouped into eight individual chapters with an index at the end.

The book begins with an article covering some of the recent advances on the biochemical nature, properties/function, and applications of invertase from a variety of sources. The discussion on protein conformational stability of the enzyme is beautifully dealt with. Following this, is a review of lactase, a commercially important enzyme in the dairy and allied industries. Lactase plays a major role in lactose intolerance and methods to alleviate the same are ably presented. The article appears interesting with the inclusion of industrial process schemes for the hydrolysis of milk and whey syrups, as well as several photographs. In the next chapter is presented a discussion on enzymes capable of hydrolysing maltose. Such enzymes, having a very wide substracte specificities, are ubiquitous in nature. Industrially they are of great commercial value in the production of glucose syrups from starch. Trehalase(s), an enzyme of bio-chemical interest is reviewed in chapter 4 in considerable length from an academic point of view. The physiological role of this enzyme is much debated. The succeeding two chapters deal with the biochemistry of several disaccharidases, including their physiological and nutritional significance. The last two chapters describe the various biochemical parameters of several disaccharides of food origin. In addition to functioning as possible energy sources these disaccharides are very much inovlved in a variety of clinical symptoms manifest in humans (and animals).

In short, the book summarizes a timely and an up-to-date account of disaccharidases, which have not been covered so far in a single book of this nature. It is impeccably edited, nicely indexed and beautifully produced. It is generally free of typographical errors. The generous list of references, as supplements to each article, should make the book a useful reference volume to food scientists and technologists as well as biochemists and nutritionists. The book is a valuable addition to the library.

R. N. THARANATHAN
C.F.T.R.I., MYSORE

INSTRUCTIONS TO AUTHORS

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

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

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4. **Abstract:** The abstract should indicate the principal findings of the paper. It should be about 200 words. It should be in such a form that abstracting periodicals can readily use it.

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

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

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

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

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

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

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

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