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(INDIA)

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RESEARCH PAPERS

Development of a Process for Preparation of Soy Paneer

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Processing parameters for preparing soy paneer such as level of soy dal to water for extraction, total solids content in soy milk, coagulation temperature and concentration of coagulant were standardised. Maximum extraction of total solids (55%) and proteins (62.7%) was when dal to water ratio was at 1:10. Soy milk (6.0% total solids) on coagulation at 75°C gave maximum yield of soy paneer. Citric acid as coagulant gave maximum yield with high content of total solids and protein in the product compared to tartaric, lactic and gluconic acid. Soy paneer prepared by the use of citric acid had 74.0% moisture, 15.5% protein and 3.9% fat. The product possessed fragile texture. However, fried soy paneer resembled milk paneer in taste, colour and sponginess. Consumer preference trials with 150 families indicated that the product was highly acceptable.

Soybeans are an excellent and cheap source of calories and quality proteins. They contain about 35-40 per cent protein and 18-20 per cent fat[1] and can therefore be useful in combating protein-calorie malnutrition in the poorer strata of population.

A variety of acceptable foods can be developed from soybeans to fit in the Indian dietary pattern. Coagulation of soy milk yields a white, soft gelatinous mass. The product has bland taste and unique body and texture resembling paneer obtained from milk in appearance and physico-chemical characteristics. Thus it can serve as a substitute for milk paneer and can also be a cheaper source of quality proteins. Besides this, development of a paneer analog will also make available milk hitherto used for paneer manufacture for consumption as fluid milk. Vijaylakshmi and Vaidehi[2] prepared acceptable products from the coagulum obtained by precipitation of soy milk or its combination with other milks.

Consequently, the present investigation was undertaken to develop a method for preparation of a paneer-like product from soybeans. The physico-chemical characteristics of the product and its consumer acceptability were also determined.

Materials and Methods

Dry mature soybeans (variety 'Bragg') obtained from Crop Research Centre of the University were used in this study. The beans were cleaned, graded and dehulled by using the dehuller of the Agriculture Engineering Department of the University. Soy dal (soybean splits) thus obtained was stored at 15°C in a closed container.

Preparation of soy milk: Soy dal was soaked in water (1:3 w/v) for 14-16 hr. The soak water was decanted and the dal washed with fresh water, and blanched for 40-45 min in boiling water and ground in a colloid mill with hot water (85-90°C); 100, 135 and 170g of dal per liter of water were used for grinding. The resulting suspensions were filtered through double layered cheese cloth and the filtrates boiled for 10 min with continuous stirring to prevent sticking of solids and scorching. Soy milk thus obtained was analysed for total solid and protein contents.

Preparation of soy paneer: Preliminary investigations were conducted to determine appropriate temperature for coagulation of soy milk. The temperatures tested were 65, 75 and 85°C. Coagulation of soy milk at 75°C yielded soy paneer with maximum protein (17.75 per cent) and total solids content (27.85 per cent). Hence, this temperature was used for coagulation of soy milk throughout the study. Lactic acid, tartaric acid, and citric acid (1.0, 2.0 and 3.0 per cent) and gluconic acid (1.0, 1.3 and 1.6 per cent) were used as coagulants for the preparation of paneer.

Soy milk was heated to the coagulation temperature and the coagulant added with gentle and continuous stirring. The contents were left undisturbed at coagulation temperature for 30 min in a water bath and thereafter, allowed to cool at room temperature. The

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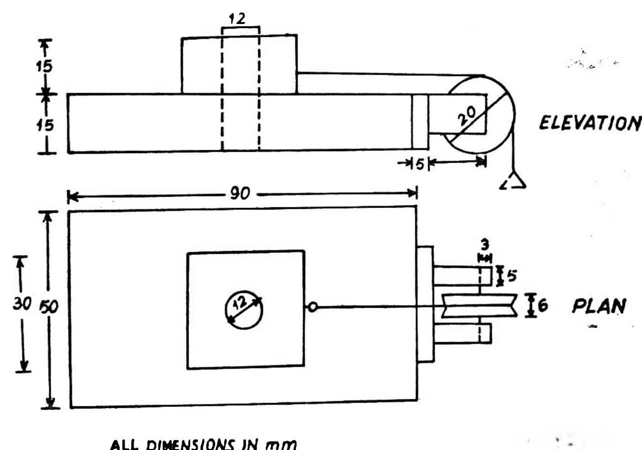

Fig 1. Shear test apparatus

whey was removed by filtration using double layered cheese cloth. The coagulum thus obtained was pressed at 1 psi to expel whey. Thereafter, it was placed in cold water for 1 hr, the water drained and stored at 4-5°C prior to use.

Deep fat frying: The soy paneer was cut into pieces of 1.5×1.5×1.0 cm and deep fat fried in hydrogenated vegetable oil at 180°C for 8 min.

Analyses: Shear strength was determined by using shear test apparatus (Fig. 1) developed and fabricated by Agricultural Engineering Department of the University. The sample was drawn by a core sampler of 1 cm internal diameter. Sample was then placed in a block of 1.2 cm diameter. By gradually increasing the weight applied, the weight just sufficient to shear off the sample was noted and shear strength was calculated as follows.

$$\text{Shear strength} = \frac{mg}{A}$$

where m denotes mass, g acceleration due to gravity and A area of the sample.

Porosity of soy paneer was calculated as follows:

$$\text{Porosity } (\eta) = \frac{V_o - V_s}{V_o}$$

Where V_o is initial volume and V_s is the volume occupied by solids in the sample.

For calculating V_s, a piece of soy paneer having mass (m) and volume (V_o) was dried and its dried mass (md) and moisture (M) were determined. V_s was calculated as follows:

$$V_s = V_o - \frac{M \times md}{100}$$

Protein content was determined by Kjeldahl method using nitrogen to protein conversion factor of 6.25. Moisture, fat, ash and calcium contents were determined according to AOAC[3] procedures. Carbohydrate content was calculated by difference.

Consumer preference trials: Fried soy paneer prepared by using 2 per cent citric acid as coagulant was used to conduct consumer preference trials. One hundred fifty samples each weighing 200 g were distributed among the consumers of the campus at random. The consumers were directed to use the product in a manner similar to milk paneer and record their preference on a hedonic scale ranging from 1 to 9, where 1 represented dislike extremely and 9 represented like extremely[4].

Results and Discussion

Soy dal contained 11.15 per cent moisture, 40.00 per cent protein and 19.50 per cent fat. Table 1 shows the effect of different levels of soy dal on yield, total solids and protein content of soy milk. A level of 100 g soybean/l of water yielded soy milk with minimum total solids and protein content. However, it gave rise to maximum extraction of proteins (62.67 per cent) and total solids (55.00 per cent). These results show that increase in the level of soy dal to water resulted

TABLE 1. EFFECT OF DIFFERENT LEVELS OF SOY DAL ON YIELD, TOTAL SOLIDS AND PROTEIN CONTENT OF SOY MILK AND RESIDUE[1]

	Soybeans		Soy milk					Residue		
Soy dal ground/l of water (g)	Total solids** (g)	Protein** (g)	Yield (ml)	Total solids (%)	Total solids recovery (%)	Protein (%)	Protein recovery (%)	Yield (g)	Total solids (%)	Protein (%)
100	88.85	40.00	820	6.0	55.00	3.00	62.67	86	15.65	5.68
135	119.95	54.00	780	7.5	48.89	3.50	50.51	118	17.00	6.78
170	151.00	68.00	700	8.8	40.87	3.93	42.42	127	17.90	7.65

[1]Average of two determinations.

**Quantities present in soy dal used.

TABLE 2. EFFECT OF DIFFERENT CONCENTRATIONS OF SOY MILK SOLIDS ON YIELD, TOTAL SOLIDS AND PROTEIN CONTENT OF SOY PANEER*

Total solids in soy milk (%)	Soy paneer			
	Yield⁺ (g)	Total solids (%)	Protein (%)	Protein (dry basis) (%)
6.0	202	25.85	15.75	56.55
7.5	183	24.50	14.00	53.42
8.8	172	22.50	11.37	50.53

Average of two determinations
*Citric acid was used as coagulant was 2%
⁺Yield per liter of soy milk.

in corresponding decrease in the recovery of soy solids and protein in soy milk. Wang[5] had reported that a level of 100 g soybean/l of water was optimum for the extraction of soy solids and protein and that high solids in the slurry yielded a more viscous suspension causing difficulty in filtration thus resulting in higher losses of solids and proteins in the residue. Extraction of proteins observed in the present investigation using organic acids as coagulant was found to be higher than that obtained by calcium sulphate (55.00 per cent)[1].

As shown in Table 2, increase in soy milk solids from 6.0 to 8.8 per cent decreased yield, total solids and protein content in paneer. Therefore, further studies were conducted with soy milk containing 6.0 per cent total solids.

Table 3 shows the effect of different coagulants and their concentrations on yield, total solids and protein content of soy paneer. Decreasing trend in all these was observed with increasing concentrations of lactic acid, citric acid or tartaric acid. In contrast, the reverse trend was observed when gluconic acid was used as coagulant. The isoelectric point of soy protein[6] is in the range of 4.2-4.6. Therefore, maximum precipitation of soy protein was observed in this pH range. Addition of one per cent lactic acid, tartaric acid or citric acid resulted in pH in the range of isoelectric point of soy proteins. Similar pH was obtained when 1.6 per cent of gluconic acid was used as coagulant.

Soy paneer obtained by lactic acid or tartaric acid or citric acid contained 74.03 to 75.38 per cent moisture

TABLE 3. EFFECT OF DIFFERENT CONCENTRATIONS OF COAGULANTS ON YIELD, TOTAL SOLIDS AND PROTEIN CONTENT OF SOY PANEER*

% concn of coagulants	Soy paneer			Whey
	Yield of soy milk (g/l.)	Total solids (%)	Protein (%)	pH
	Lactic acid			
1.00	182	26.80	16.40	4.32
2.00	168	24.90	15.94	3.54
3.00	153	22.40	14.26	3.09
	Citric acid			
1.00	211	28.00	16.37	4.38
2.00	202	26.12	15.75	4.02
3.00	186	25.60	15.31	3.89
	Tartaric acid			
1.00	187	27.80	16.93	4.22
2.00	173	25.60	14.43	3.92
3.00	159	23.50	13.31	3.48
	Gluconic acid			
1.00	142	17.50	10.93	4.90
1.30	149	18.80	11.15	4.76
1.60	159	19.75	11.68	4.28

*Average of two determinations
*Soy solids in milk was 6.0 per cent.

TABLE 4. PROXIMATE COMPOSITION OF UNFRIED AND FRIED SOY PANEER OBTAINED BY USING CITRIC ACID OR GLUCONIC ACID AS COAGULANT*

Coagulant used	Paneer type	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)	Calcium (mg/100g)
Citric acid	Unfried	74.00	15.53	3.88	1.24	5.32	101
	Fried	33.50	24.71	28.67	1.41	11.71	210
Gluconic acid	Unfried	81.20	11.15	2.94	0.84	3.87	93
	Fried	36.00	21.65	31.71	0.98	9.66	188

*Average of two determinations
The concentrations of citric acid and gluconic acid used were 2.00 and 1.3 per cent, respectively.

TABLE 5. EFFECT OF DIFFERENT CONCENTRATIONS OF COAGULANTS ON THE PHYSICAL CHARACTERISTICS OF UNFRIED AND FRIED SOY PANEER*

Coagulant concn (%)	Unfried soy paneer			Fried soy paneer		
	Moisture (%)	Porosity	Shear strength (dynes/cm^2 x 10^4)	Moisture (%)	Porosity	shear strength (dynes/cm^2 × 10^4)
			Lactic acid			
1.00	73.30	0.23	3.93	32.25	0.53	15.73
2.00	74.20	0.29	3.50	33.90	0.60	15.11
3.00	76.62	0.32	2.92	34.40	0.62	14.36
			Citric acid			
1.00	72.86	0.17	4.00	30.70	0.47	14.80
2.00	73.60	0.22	3.87	33.50	0.57	13.80
3.00	74.63	0.44	3.74	35.30	0.65	12.55
			Tartaric acid			
1.00	72.10	0.37	6.93	30.30	0.53	16.04
2.00	74.22	0.38	5.06	33.60	0.58	15.73
3.00	76.33	0.38	4.49	34.65	0.58	13.86
			Gluconic acid			
1.00	82.50	0.46	2.93	38.50	0.57	14.05
1.30	81.20	0.37	3.09	36.00	0.54	14.82
1.60	80.25	0.35	3.30	33.00	0.52	15.11

*Average of two determinations
*Solids in soy milk 6.0 per cent.

and 15.53 to 15.75 per cent protein. In contrast, gluconic acid yielded a product with higher moisture (81.20 per cent) and lower protein (11.15 per cent) content (Table 4). Frying the product increased all the constituents substantially. The increase in fat content may be ascribed to absorption of fat and expulsion of moisture during frying whereas increase in other constituents was presumably because of expulsion of moisture during frying.

The shear strength of paneer decreased with increasing concentrations of lactic acid, or citric acid or tartaric acid used as coagulant (Table 5). However, when gluconic acid was used the shear strength increased with the concentration of acid. This increase in shear strength of gluconic acid coagulated paneer was due to the higher moisture level as compared to the paneer obtained with other coagulants. Thus, it can be concluded that the shear strength of paneer is indirectly proportional to its moisture content. As expected the porosity of the product was directly correlated to its moisture content. Frying of the product increased the shear strength and porosity in all the cases.

Preliminary trials were conducted to determine acceptability of soy paneer prepared by using different concentrations of coagulants. Use of 2 per cent citric acid as coagulant yielded a product with the highest acceptability score. Hence, this concentration was used to prepare paneer for consumer preference trials. The product was highly acceptable, the score being 16.7 per cent like extremely; 50 per cent like very much; 33.3 per cent like moderately.

The results of this investigation demonstrate that an acceptable product resembling milk paneer in appearance, taste and texture can be prepared by coagulation of soy milk with organic acids such as citric acid.

Acknowledgement

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The Occurrence of *Aspergillus flavus* and Aflatoxins in Indian Cottonseed*: A Survey

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A survey was carried out on the presence of *Aspergillus flavus*, the occurrence of greenish-yellow fluorescence (GYF) and aflatoxins in cottonseed samples from dry and humid regions in India. In the humid areas, 93% of the samples were infected by *A. flavus*, 81% of the samples showed fluorescent seed, averaging 23 GYF seed/kg. Twenty seven per cent of the samples contained aflatoxins in the range 51-500 μg/kg and 16% contained $>$ 1000 μg/kg. In the dry area most of the samples had relatively low incidence of *A. flavus;* GYF averaged 3.2 seeds/kg and aflatoxins were found at levels from 51-500 μg/kg only in 9% of the samples. Sixty nine per cent of the samples from the dry region were free from aflatoxin. Aflatoxin B_1 and B_2 were the only aflatoxins found in the naturally contaminated cottonseed.

Aspergillus flavus Link is an important fungal contaminant of cotton and damages the seed and fiber[1]. Certain strains of this fungus are known to elaborate aflatoxins in cottonseed[2]. Although *A. flavus* is principally a post-harvest invader of stored crops[3] it can infect partially—open and moist cotton bolls and hence the cottonseed in the standing crop. The raw cotton thus infected sometimes shows a yellow discoloration and under ultra violet light the cotton or the ginned seed shows spots with a bright greenish-yellow fluorescence (GYF).

The presence of GYF in cottonseed was associated with the invasion of the bolls by *A. flavus*[1,4] and it was later shown that peroxidase in the immature bolls reacted with kojic acid, a metabolite produced by *A. flavus*, to give compounds that fluoresced greenish-yellow[5]. Further work showed an association between the degree of development of GYF and the concentration of aflatoxins in the seed which suggested the possibility of reducing aflatoxin contamination by screening and removal of GYF seeds by electronic sorters[4,6,7]. Ashworth *et al.*, [8–11] have reported that in Southern California, the 1967 harvest contained a high percentage of seed which were invaded before harvest by fungi, including *A. flavus* and showed the presence of aflatoxins. However during storage, seed infection by *A. flavus* increased but there was no increase in aflatoxins. The danger to animal health presented by aflatoxins is now widely recognised. Further, milk from milch animals consuming mycotoxin contaminated feeds has also been shown to contain aflatoxin M_1 and other modified forms of aflatoxin B_1. These modified aflatoxins have toxicity comparable to aflatoxin B_1, a most toxic component of the aflatoxin group[12].

While some countries have stipulated zero-tolerance for aflatoxins in foods and feeds, many others have limits ranging from 5 to 50 ppb. Countries like Japan were permitting imports of groundnut and cottonseed meals containing 1 ppm for animal feeds but it is understood that now stricter limits are being imposed.

This communication reports the data on the seed-borne fungi, and particularly the occurrence and distribution of *A. flavus*, the occurrence of GYF seeds, and the production of aflatoxins, in cottonseed samples collected from two different climatic regions in India.

Materials and Methods

Seed samples from dry areas (Maharashtra, Andhra Pradesh and Gujarat) were collected where cotton is grown as a rainfed crop (during and after the monsoons: August to March) and from humid coastal areas (Krishna and Guntur districts of Andhra Pradesh, where it is grown as an irrigated crop in rice fallows (December to June, before the rains). The samples ($\sim$ 2 kg) were collected from various gins, oil mills,

*This paper is based on the work done and reported under a PL 480 Project (FG-In-342) during 1967-1970.

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agricultural research stations and from the standing crop. The details of sample collection are as follows.

Samples were collected from the following dry areas.

Maharashtra state: Totally 125 samples were collected from Nanded-Akola-Amraoti sector, during January-December 1968 and February 1969. The crops were either rainfed or irrigated. Samples were from stored heaps ($\frac{1}{2}$-1 year old), rain damaged, stored bags (one year old) and standing crop (bolls). The temperature range was 25-30°C during Jan-March and 20-25°C in December. Humidity in March-April was 30 per cent, in October 60 per cent and in June-Sept. more than 60 per cent.

Andhra Pradesh: Totally 108 samples were collected from Adilabad sector and Nandyal-Adoni sector during January-March 1968. Sample collection was done from rainfed crop (bolls) (hard locks), stored bags (1 and 3-5 year old) and heaps ($\frac{1}{2}$-1 year old) rain damaged. Average temperature was 25-35°C and humidity during June to December was 70-75 per cent and other periods 55 per cent.

Gujarat: Nineteen samples were collected from Surat sector from stored heaps in February 1970. The temperature was 30-35°C and humidity was less than 65 per cent.

Karnataka: Thirty samples were collected from 2-5 months old bags from Raichur-Gadag-Davanagere sector in April 1970. The average temperature was 20-25°C and humidity less than 65 per cent.

The humid area chosen was Masulipatam-Guntur sector of Andhra Pradesh. Totally 124 samples were collected in August 1968, April, May, June, Oct. 1969 and August/Sept. 1970. The samples were from stored bags (1-2 year old), stored heaps (one month old) and rain damaged and damaged seed bolls. Temperature was 30-35°C and humidity was May-June 65-70 and 75-85 per cent during other periods of the year.

Seed-borne infection by *A. flavus* and other fungi was determined as follows: The seeds were delinted with concentrated sulphuric acid, and washed free of acid with tap water, followed by three washes with sterile distilled water. They were then surface sterilized with 0.1 per cent aqueous mercuric chloride for 1 min and washed four times with sterile distilled water. Surface-sterilized seeds (100 seeds; 4 per petri dish) were plated on potato-dextrose agar (containing 6 per cent sodium chloride, 0.1 per cent sodium tauroglycocholate and 0.01 per cent streptomycin sulphate). The plates were incubated at room temperature (27-30°C) and observations recorded after 7 days. The number of seeds showing fungal growth was recorded and their percentage calculated. The fungi developing on the seed were brought under pure culture and identified to genus. In case of *Aspergillus* spp. specific and group identifications were made [3].

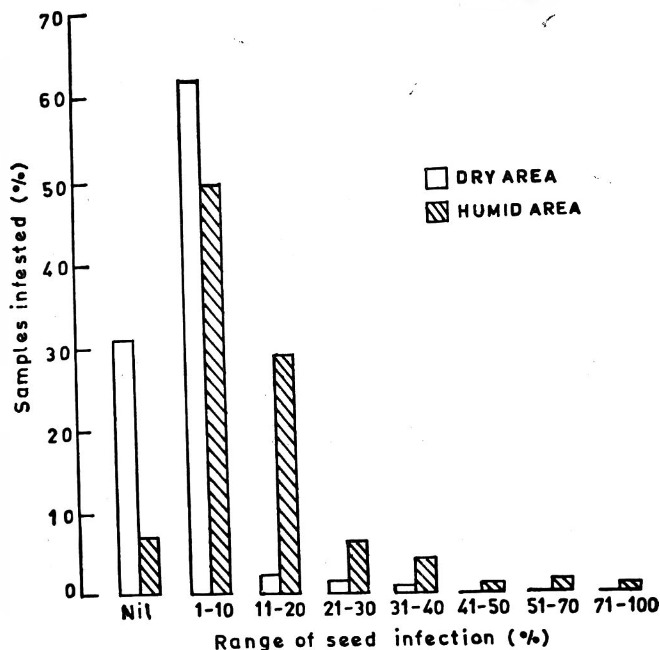

Fig 1. Distribution of *A. flavus* in infected cottonseed

The GYF seeds were estimated by spreading ~1 kg in an enamelled tray and counting the seeds which showed greenish yellow fluorescent spots under a long-wave (366 nm) ultraviolet lamp. The cottonseed kernels, obtained by dehulling the seed in a small turn roll mill were estimated for aflatoxins by the procedure of Pons *et al.*[14] The identity of aflatoxins was confirmed by co-chromatography with a standard mixture of the four aflatoxins and by development in three solvent systems: chloroform-acetone (85:15), toluene-ethyl acetate-90 per cent formic acid (5:4:1), and benzene-methanol-acetic acid (24:2:1).

Results and Discussion

Cottonseed infected with A. flavus: From Fig. 1, it can be seen that in the dry areas 31 per cent of the samples were free of *A. flavus*, about 63 per cent of the samples carried seed infection at the level of 1–10 per cent and only a small percentage (~6%) of samples showed infection range<10 per cent. On the other hand, in the samples from the humid area only 7.2 per cent were free of the fungus; and of the infected samples, nearly half were in the infection range of 1–10 per cent and 29 per cent in the 11–20 per cent range.

Aflatoxin in cottonseed: Data in Fig. 2 show that only 22 per cent of the samples from the humid areas were free from aflatoxin while those from dry areas were 68.5 per cent. The number of samples from the dry areas containing aflatoxin in this range was 27.7 per cent (19% in the 1–50 ng/kg range and 8.7 per cent in the 51–500 ng/kg and 38 per cent>1000 ng/kg).

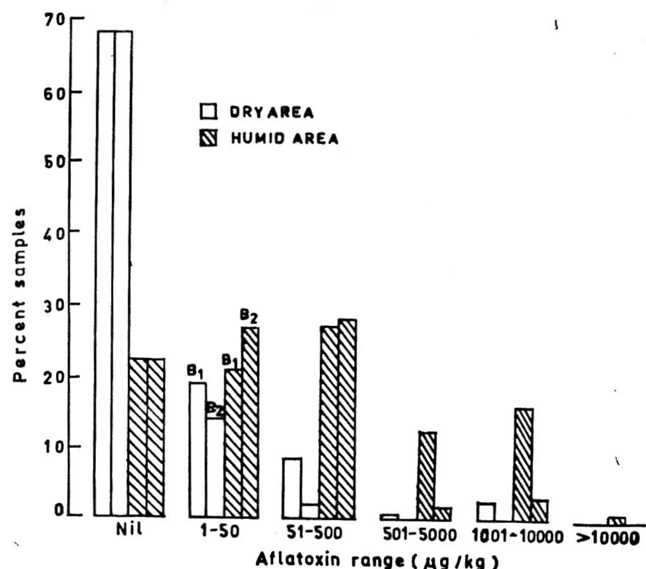

Fig 2. Distribution of aflatoxins B_1 and B_2 in cottonseed

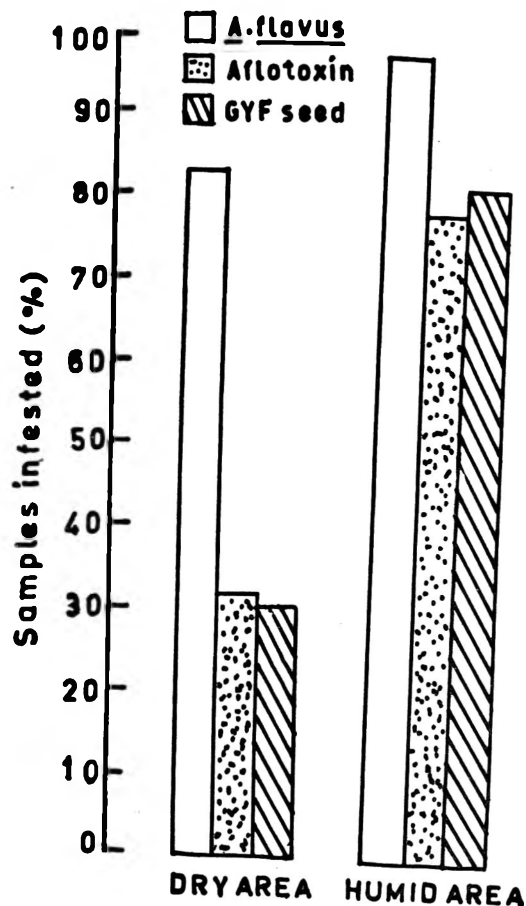

Fig 3. Correlation between the presence of *A. flavus*, aflatoxin and GYF seed.

It is interesting to note that aflatoxins G_1 and G_2 were never detected in all the samples analyzed in this work. The toxigenic isolates of *A. flavus* were grown on rice and analysed for aflatoxins. Except for one sclerotial isolate which produced all the four aflatoxins namely B_1, B_2, G_1 and G_2 all the other isolates produced only B_1 and B_2.

A. flavus, *GYF seed and aflatoxins*: Fig. 3 shows that there is some correlation between the percentage of samples showing aflatoxin producing strains of *A. flavus*, and samples containing aflatoxin as well as the samples showing GYF seed, but this was more evident in samples from the humid area than in the dry area. Thus about 69 per cent of the samples from the dry area were observed to be infected but only 30 per cent contained aflatoxin and 31 per cent were GYF. On the other hand, of the 93 per cent of the samples observed to be infected from the humid area, 78 per cent showed the presence of aflatoxin and 81 per cent were GYF.

The present investigation has clearly shown the predominance of *A. flavus*, GYF seeds, and aflatoxins in seeds from the humid area. The types or species of fungi which predominated are attributed mainly to the differences in environmental factors. This will be reported separately. Such environmental factors are important particularly in the rice-cotton crop rotation under irrigation in the humid coastal areas. Although the cotton crop matures during the summer months, the average humidity is $\sim$ 75–85 per cent for a greater part of the year except for a short period in May when high temperature and low humidity (about 65%) prevailed.

A small number of samples analysed for moisture indicated 9–13 per cent in seeds from the humid area and 7–9 per cent in seeds from dry areas. In humid areas, the moisture content is likely to be higher when the seed is ginned and stored, especially just before or during the monsoon, the high incidence of both *A. flavus* and the GYF seed in cottonseed grown in rice-cotton rotation suggests that the humidity was high in the irrigated fields, especially in the vicinity of the shaded portion of the plants. Under such conditions, bolls may open more slowly, because they take longer to dry, and thus the insufficiently dried cotton fibre would be prone to infection by *A. flavus*.

The occurrence of high levels of GYF in humid areas, confirms the infection of bolls. This primary infection[10] would lead to subsequent spread to the seed, if humid conditions continued. Our data showing a high incidence of *A. flavus* and aflatoxins in the seed from humid areas confirm this.

In the dry area nearly 70 per cent of the samples were free from aflatoxins, whereas about 78 per cent of those from humid areas contained aflatoxins and

about 25 per cent of these contained above 500 μg/kg. Generally the production of aflatoxin B_1 under natural conditions was accompanied by the elaboration of afllatoxin B_2, both these toxins were found in a greater proportion in the samples of humid areas. The data also show that in naturally contaminated cottonseed, aflatoxins G_1 and G_2 were never encountered. It is also significant that *A. parasiticus*, known to produce all the four toxins, was not encountered in the 2866 isolates of *A. flavus* group.

The three parameters namely, the frequency of *A. flavus* infection, GYF seed and aflatoxin content show a close relationship in the humid areas, and to a lesser degree in the dry areas. GYF seeds found in the samples from the humid areas averaged to 23/kg cottonseed, while in samples from the dry area this average was 3.2 seeds/kg cottonseed. The presence of aflatoxin and GYF seed appears to be very closely related (Fig. 3) in samples from dry as well as humid areas. If this is so then the lower number of GYF seeds in the dry areas may be due to the large number of samples showing only a low level of infection by *A. flavus*, and the dry conditions precluded further growth and aflatoxin production. In the humid areas there is apparently a large scale infection by the fungus during the boll opening. The wet conditions subsequently may promote the growth of *A. flavus* and the production of aflatoxin as well as GYF seed.

The study shows the need for surveys of the aflatoxin producing fungi and the analytical data on aflatoxin content in cottonseed with reference to the environmental conditions during the growth of the crop.

Acknowledgement

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Texture Characteristics of Cassava—Wheat Dough and Sensory Properties of Chapathi

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Texture qualities of cassava and wheat flour and their blends were studied. Parboiling of fresh cassava slices increased firmness and cohesiveness of the dough. Incorporation of plain cassava flour to wheat flour at levels of 30% and above improved the colour and appearance of chapathi, but scored less in handfeel, mouthfeel, and taste compared to chapathi prepared from whole wheat flour. Blending of parboiled cassava flour with wheat flour improved the organoleptic qualities of the product.

Cassava is a major food crop of tropics. Acceptability of this tuber depends upon the form in which it is consumed[1]. Though the suitability of cassava flour for bread-making has been explored by different workers[2,3], no information is available on the texturometer studies of the dough prepared from either plain or parboiled cassava flour or a blend of wheat and cassava flours. In the present paper, results of the textural studies conducted with wheat, cassava and wheat-cassava dough samples and evaluation of organoleptic qualities of chapathi prepared from them are discussed.

Materials and Methods

Freshly harvested cassava tubers of cultivar 'Malayan-4' (M-4) were peeled and sliced to chips of thickness 4-5 mm.

Preparation of dried chips: For parboiling, fresh slices of 1.0 kg lots were soaked in boiling water for 5 min, and the water drained. The parboiled and plain fresh slices were spread in aluminium trays (80×41 ×3 cm) in monolayers and dried in a cross-flow dryer at 58±2°C for about 8 hr. The plain and parboiled chips had 9-10 and 8-9 per cent moisture respectively. Dried chips were ground in a plate mill to pass through a 60 mesh sieve (250 μ).

Preparation of wheat flour: Wheat of medium-hard quality was procured, cleaned and milled in a plate mill. The whole wheat flour had a moisture of 9-10 per cent.

Texture studies: Textural characteristics were studied using a General Foods Texturometer (Zenken Co., Japan). Firmness, cohesiveness and stickiness were measured with individual samples and with wheat-cassava blends. Wheat-cassava flour blends of (1) 3:7, (2) 1:1 and (3) 7:3 were employed. The above ratios were selected randomly so as to get (1) low, (2) equal and (3) high content of either of the two individual components in the blend.

Preparation of dough sample: To 50 g dough a definite volume of water, arrived at from standardisation trials, was added and mixed in a mechanical mixer; a dough of smooth external appearance resulted within 2 min. Ten g of dough was smoothly rounded by hand and after a resting time of 10 min texturometer readings were taken.

A flat plunger of 50 mm diameter was attached to the instrument. The other instrumental conditions were: (*a*) voltage-2v, (*b*) speed-low i. e. 6 bites per min, (*c*) clearance—2 mm and chart attenuation $\frac{1}{5}$. Five replications were done and the mean value along with standard deviation are presented.

Organoleptic studies: Chapathis prepared from wheat-cassava dough were compared with control wheat chapathis by a seven member panel, initially familiarised with the qualities to be evaluated viz. colour, texture (hand and mouthfeel) appearance and taste. The method of scoring was essentially that of Hopkins[4] but with a score of 10.0 being considered 'ideal'.

Preparation of chapathi: For making chapathi, 50 g of each flour was kneaded to dough after adding a definite volume of water and ingredients like salt and coconut oil. The dough was hand-rolled to chapathi of thickness not more than 1 mm and diameter about 15 cm. It was directly placed on a hot gridle (*Tawa*) and roasted to get a uniformly puffed product, the time required being about 3 min. It was cut into small triangular pieces and transferred to petri-dishes. Each sample was coded to prevent the identification of the

TABLE 1. TEXTUROMETER VALUES* OF DOUGH BASED ON WHEAT AND CASSAVA FLOURS

WWF	PCF	PBCF	Water added (ml)	Firmness (H) (kg/v)	Cohesiveness (A_1/A_2)	Stickiness (–H) (kg/v)
100	—	—	46	5.55±0.14	0.66	1. 5±0.24
—	100	—	40	6.94±0.47	0.39	Nil
—	—	100	50	8.13±0.32	0.54	Nil
70	30	—	38.6	12. 7±0.47	0.58	1.66±0.48
50	50	—	39.0	11. 5±0.66	0.49	2.58±0.38
30	70	—	39.5	7. 5±0.20	0.44	1.09±0. 1
70	—	30	41.6	11. 5±0.20	0.58	3.69±0.68
50	—	50	44.0	12. 5±0.43	0.58	5. 5±0.17
30	—	70	46.5	8.06±0.42	0.57	1.21±0.27

*Mean value of five measurements for dough prepared from 50 g flour

WWF: Whole wheat flour; PCF: Plain cassava flour; PBCF: Parboiled cassava flour.

product. Three trials were conducted and the average score of seven panelists was calculated.

Results and Discussion

The results of the texture studies are presented in Table 1. Among the doughs prepared from single component, the parboiled cassava flour dough scored highest values for firmness and water absorption. The higher firmness observed could be due to the pregelatinisation of starch during parboiling. The cohesiveness value of the dough prepared from the parboiled cassava flour (0.54) was also higher than that of plain cassava dough (0.39) and the value came nearer to that of wheat dough (0.66). As cohesiveness is an index of the strength of the internal bonding of the food components[5] it is inferred that parboiling of fresh cassava slices prior to drying improves the strength of the material. Improvement in the strength of cassava chips by parboiling has already been reported by us[6]. Pregelatinisation followed by reassociation or retrogradation of starch taking place during preparation of parboiled chips could presumably be responsible for increased rehydration as well as dough cohesiveness.

Texturometer characteristics of the dough prepared from whole wheat flour got altered by incorporating cassava flour. In wheat-cassava flour dough the amount

TABLE 2. ORGANOLEPTIC EVALUATION* OF CHAPATHI PREPARED FROM WHEAT-CASSAVA FLOUR BLENDS**

WWF	PCF	PBCF	Water added (ml)	Colour	Appearance	Texture		Taste
						Hand feel	Mouth feel	
100	—	—	38.0	10.00	10.00	10.00	10.00	10.00
70	30	—	33.5	11.85	11.57	9.43	8.72	9.58
50	50	—	35.0	10.57	10.42	8.86	7.86	8.43
30	70	—	39.0	11.42	10.42	9.72	9.00	9.29
70	—	30	45.5	10.85	10.57	11.14	11.42	10.57
50	—	50	50.0	11.85	12.00	11.14	11.00	11.14
30	—	70	47.0	10.85	11.14	11.85	11.28	11.28

**Score scale:* 10 base scoring mean value for three trials, **Weighing 50 g and containing 1g salt and 1 ml coconut oil.

Score rating: +1 slight excess, +2 moderate excess, +3 decided excess, +4 very decided excess, +5 gross excess.
–1 slight deficiency, –2 moderate deficiency, –3 decided deficiency, –4 very decided deficiency, –5 gross deficiency.

of water absorbed during dough-mixing increased depending upon the amount of cassava flour present in the blend; this increase was relatively less in the samples prepared from wheat and plain cassava flour. The firmness and cohesiveness values decreased with the proportion of plain cassava flour present in the blend. In samples prepared from wheat and parboiled cassava flour cohesiveness was almost identical, while firmness value showed an increase when proportion of cassava flour increased from 30 to 50 per cent. However, further increase (i. e. 70%) resulted in lowering the firmness value. This may be due to the inability of such samples to produce sufficient number of linear matrices to form a continuous phase in which starch could be embeded to form a dough. It was also noticed from the texturometer studies that incorporation of either plain or parboiled cassava flour with wheat flour increases the stickiness of the dough the optimum value being for the blend which contained equal amounts of wheat and cassava flour.

Organoleptic studies (Table 2) indicated that colour and appearance of the chapathi got slightly improved by incorporating either plain or parboiled flour. However, chapathi prepared from wheat and plain cassava dough scored less than the control sample prepared from whole wheat flour in texture and taste. In all quality aspects chapathis prepared from wheat and parboiled cassava flour blends were somewhat better than controls.

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Pilot-Scale Storage Tests on the Efficacy of Gamma Irradiation for Sprout Inhibition of Onions Under Commercial Storage Conditions

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Storage trials under ambient conditions in a commercial onion storage facility at Pimpalgaon, Nasik Dt. in Maharashtra showed that losses as high as 70% on account of sprouting (17%), rotting (25%) and desiccation (28%) may occur in 'Rabi' onions stored in a poorly ventilated conventional 'chawl' over a period of 4–5 months. Storage in a well aerated model store reduced the total losses to 50% mostly due to a reduction in microbial spoilage. Sprouting and rotting were considerably lower in 'Rangda' onions due to the prevailing low ambient temperature and humidity conditions, although the losses were comparatively higher in the chawl. Gamma irradiation to 60 to 90 Gy inhibited sprouting regardless of the crop season, environmental conditions or the type of storage structures used. In 'Rabi' onions, which is stored commercially, irradiation followed by storage in the model store resulted in a 20% increase in marketable grade onions at the end of 4.5 months, while in the 'chawl' no beneficial effect was seen. Dry matter, soluble solids, reducing sugars, colour, pungency and flavour strength were not affected by irradiation, though an internal discolouration limited to a very small area was seen due to the dead meristem or internal sprouts.

Sprouting is one of the major causes for qualitative as well as quantitative deterioration of stored bulb onions. Sprouting leads to transfer of both dry matter and water from the edible fleshy scales into the sprouts

resulting in increased shrivelling and loss of market quality of such bulbs. While low temperatures (5 to 20°C) and high relative humidities (RH 80% and above) induce dormancy break and sprout growth, temperatures of 30°C and higher can prolong dormancy and prevent sprouting[1,2]; however, desiccation and rotting can be major loss factors at higher ambient temperatures[3,4]. Increased sprouting can also occur under widely fluctuating diurnal temperatures[2].

Ever since the report of Dallyn and associates[5] on complete inhibition of sprouting in onions by exposure to low doses of gamma radiation, several investigations have been carried out throughout the world on the application of irradiation for sprout inhibition of different cultivars and strains of onions grown under varying agro-climatic conditions, the results of which have been reviewed recently[6]. Our earlier investigations under laboratory as well as field storage conditions have shown that good sprout control in 'Nasik Red' and 'N-53' onions, the principal cultivars grown in Nasik District of Maharashtra State, could be obtained after treatment with 60 to 90 Gy of gamma rays[2,7]. However, the efficacy of gamma irradiation for sprout inhibition of onions under actual commercial storage conditions has not been evaluated so far.

The studies reported here had three major objectives: (*i*) to assess the various causative factors and their relative contributions to losses in onions stored at ambient conditions in a commercial storage facility located in Pimpalgaon, Nasik District, (*ii*) to test the effectiveness of gamma irradiation for sprout control under commercial storage conditions and (*iii*) to evaluate the overall storage performance of irradiated onions during commercial storage.

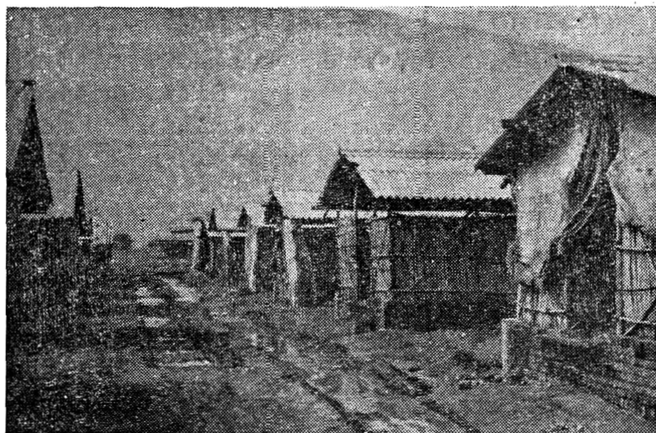
Materials and Methods

Storage trials were carried out during 1981 and 1982 seasons with Rabi onions (variety 'Nasik Red', harvested from April to May) which is commercially stored in Nasik Dt. from May-June until October. In addition one trial with Rangda crop (variety 'N-53' harvested in January-March) was conducted during 1982 season. Commercially Rangda crop is not stored in Nasik Dt. Onions were procured from Nasik district, soon after harvest and field curing was practiced by local growers.

Irradiation: Onions filled in aluminium containers in 5 kg lots were irradiated to a minimum dose of 60 Gy in a cobalt-60 Package Irradiator in air at an ambient temperature of 25-26°C at a dose rate of 12.5 Gy/min. The dose variation within the container area of 12in.×6in.×6in. size was between 30 and 40 per cent. Transport of onions packaged in jute bags to the irradiation facility in Trombay and back to the storage facility of the National Agricultural Co-operative Marketing Federation of India Ltd., (NAFED) located at Pimpalgaon Baswant, Nasik Dt., a distance of 250 km, was by truck.

Storage: Two types of storage structures were used for these studies viz. the traditional storage shed locally known as Chawl and a model store built by NAFED, both using natural ventilation (Fig. 1). The experimental samples were stored in 0.5 or 1 ton lots in replicates of 4 or 5 to a depth of 3.5 to 4.5 feet.

At the end of storage, bulbs were sorted into sprouted, rotted and marketable; the marketable bulbs were further sorted into first and second grade qualities



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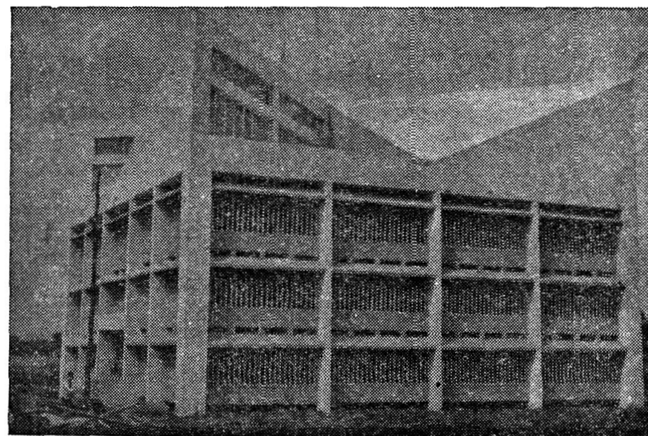

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Fig. 1.A. Conventional storage shed called *chawl* which is traditionally used by farmers and traders for storage of onions in Nasik Dt. in Maharashtra. Onions are stored 4 to 5 feet high on raised earthern platforms 12 to 18 in. above ground level. Dry twigs of Arhar plants are spread on the earthern platforms to avoid direct contact of bulbs with the soil. Side walls are made of bamboo. *Chawls* provide poor ventilation and also lack ventilation from beneath the onion pile.

Fig. 1.B. A model store built by NAFED. This is a three tier reinforced cement concrete structure with slatted platforms and side walls which provide very good ventilation through all sides of the onion pile.

based on general appearance and colour of the outer scales. The difference in the initial weight and the total weight of onions remaining at the end of storage was taken as desiccation losses.

Chemical and quality evaluation: Bulbs were cut vertically into 4 equal parts and one quarter each from 10 separate bulbs were chopped and mixed thoroughly, from which known weights were taken for various chemical analyses. Total soluble solids in the crushed juice of a composite sample were recorded with a hand refractometer and expressed as per cent sucrose. Reducing sugars in the 85 per cent aqueous ethanol extracts obtained after soxhlet extraction was estimated colorimetrically[8]. Pungency was assessed by head space gas analysis where di-n-propyl disulphide (DPDS) the major pungent principle of onions was evaluated by measuring its peak area after gas chromatographic separation[9]. The overall flavour potential was assessed by the spectrophotometric estimation of the enzymatically formed pyruvic acid in cut onions[9].

Evaluation of losses due to microbial spoilage: Bulbs affected by various diseases like black mold, basal rot, white rot and soft rot were sorted out and their weights recorded. The causal organisms were identified based on their characteristic symptoms[10] and confirmed by laboratory tests for pathogenicity.

Results

Influence of storage structure on losses: Storage losses were considerably high in the conventional *chawl* as compared to the model store (Table 1). In the 1981 season the total losses due to sprouting, rotting and desiccation in nonirradiated Rabi onions were 70 per cent in the *chawl* during 4.5 months as against 51 per cent in the model store. A similar trend was also seen in the 1982 trials. While sprouting and desiccation losses were comparable under both types of storage, rotting was very heavy in the *chawl.* This increased incidence of microbial spoilage in the *chawl* was evident even when the height of the onion pile was reduced to 1.5 feet in one experiment, although lowering of the bulk height generally reduced the total losses in both *chawl* and model store (Table 1). With nonirradiated Rangda onions, the losses occurring in both *chawl* and model store on account of sprouting and rotting were considerably lower than that observed in rabi onions whereas desiccation losses were of a similar magnitude (Table 2).

Irradiation effect on sprouting and total losses: Irradiation resulted in complete suppression of sprouting in Rabi and Rangda onions regardless of the time of irradiation viz. two or three weeks after harvest. Apart from sprout inhibition, irradiation generally decreased the losses due to desiccation especially in the model store. Thus in the 1981 rabi experiment in the model store, losses due to desiccation in nonirradiated onions amounted to 28 per cent as against 15 per cent in the irradiated lots (Table 1). After 4.5 months of storage, 72 per cent of the initial quantity of the irradiated onions were of marketable grade as against only 49

TABLE 1. COMPARATIVE STORAGE PERFORMANCE OF GAMMA IRRADIATED AND NONIRRADIATED 'RABI' ONIONS UNDER COMMERCIAL CONDITIONS*

Type of storage	Treatment	Total marketable grade (%)	First grade (%)	Second grade (%)	Total losses (%)	Sprouted (%)	Microbial spoilage (%)	Desiccation (%)
		After 4.5 Months Storage, June-Oct. 1981						
Model store	Control	49.0±5.6	18.2	30.8	51.0	21.1±1.7	2.1±3.3	27.7±5.4
	Irradiated	72.6±10.2	26.7	45.9	27.4	Nil	8.8±6.6	15.2±7.4
	Control**	63.0				10.0	1.0	26.0
Chawl	Control	29.7±5.4	10.6	19.2	70.3	17.4±6.4	24.5±5.6	28.4±4.2
	Irradiated	35.7±8.6	14.8	20.9	64.3	Nil	34.2±0.1	30.1±3.1
	Control**	44.0				12.0	22.0	22.0
		After 3.5 Months Storage, June-Oct. 1982						
Model store	Control	57.6±6.8	35.2±3.2	22.0±7.5	42.4	17.0±1.5	6.1±5.7	18.3±1.3
	Irradiated	76.9±4.9	52.6±4.9	24.4±3.3	23.1	Nil	4.4±3.0	17.5±3.3

*The data are mean and standard deviation as a percentage of the initial quantity stored. In 1981 trials onions were stored in 0.5 ton lots (5 replicates) and in 1982 trials in 1 ton lots (4 replicates).

**Onion bulk height 1.5 feet only as against 3.5–4.5 feet in others. Data based on a single lot of 1 ton.

TABLE 2. COMPARATIVE STORAGE PERFORMANCE OF GAMMA IRRADIATED AND NONIRRADIATED RANGDA ONIONS UNDER COMMERCIAL CONDITIONS*

Type of storage	Treatment	Total marketable grade (%)	First grade (%)	Second grade (%)	Total losses (%)	Sprouted (%)	Microbial spoilage (%)	Desiccation (%)
Model store	Control	68.7±4.2	31.4±3.7	37.3±3.3	31.3	2.3±0.5	1.8±0.4	27.2±3.5
	Irradiated-A	71.8±5.4	33.5±5.0	38.3±6.2	27.2	Nil	3.1±1.0	25.1±4.6
	Irradiated-B	74.0±4.0	31.8±5.7	41.9±2.7	26.0	Nil	3.1±0.7	22.9±4.5
Chawl	Control	59.4±8.0	26.6±6.6	32.8±5.1	40.6	10.8±5.5	7.1±3.5	22.0±2.5
	Irradiated-A	73.9±2.1	24.7±5.9	51.9±2.9	26.1	Nil	7.6±2.9	17.6±2.2
	Irradiated-B	71.9±4.2	28.4±11.1	43.2±7.5	28.1	Nil	12.0±3.8	15.6±3.8

*The data are mean and standard deviation expressed as a percentage of the initial quantity stored. Based on 4 replicates of 0.5 ton each in each treatment.

A-onions irradiated 2 weeks after harvest; B-onions irradiated 3 weeks after harvest.

(Storage duration: 3.5 months, January-May, 1982)

per cent in non-irradiated onions. A similar increase (19 per cent of the original weight) in marketable grade onion was observed in irradiated rabi onions in the 1982 experiments, although the differences in the desiccation rates were not apparent. Also irradiated onions recorded a higher percentage of first grade bulbs in comparison to nonirradiated lots. The beneficial effect of irradiation in terms of sprout control and desiccation was less pronounced in the *chawl* possibly due to the increased microbial rotting in *chawl* storage. A reduction in desiccation rate and an increase in total marketable grade onions by irradiation, but of a lesser magnitude, was also seen with the Rangda onion (Table 2).

Irradiation effect on microbial spoilage: The pattern of microbial spoilage shown in Table 3, indicates that both in *chawl* and model store, the incidence of bacterial soft rot was comparatively higher in irradiated bulbs whereas black mold predominated in nonirradiated bulbs.

Irradiation effect on quality: No appreciable differences in the colour of outer dry scales and the inner fleshy scales as well as in the content of dry matter, soluble solids, reducing sugars, pungency and overall flavour potential of onions were discernible during storage as a consequence of irradiation (Table 4).

A brownish internal discolouration limited to a very small area near the basal end was observed in irradiated onions when the bulbs were cut open (Fig 2). This discolouration was noticeable initially after 3 to 4 weeks of irradiation and its intensity appeared to increase with advancing storage period.

TABLE 3. MICROBIAL SPOILAGE PATTERN (% SHARE OF LOSSES DUE TO DIFFERENT SPOILAGE ORGANISMS) IN RABI ONIONS STORED UNDER COMMERCIAL CONDITIONS AS INFLUENCED BY IRRADIATION AND TYPE OF STORAGE

Spoilage organism	*Chawl*		Model store	
	Control	Irradiated	Control	Irradiated
Black mold (*Aspergillus niger*)	64.3±18.4	57.2±10.5	71.3±23.3	44.2±14.7
White rot (*Sclerotium rolfsii*)	0.27	Nil	0.05	Nil
Bacterial soft rot (*Erwinia* spp.)	34.6±18.6	41.9±10.0	28.1±23.3	55.4±14.7
Basal rot (*Fusarium* spp.)	0.80	0.93	0.34	0.24

TABLE 4. CHANGES IN DRY MATTER, TOTAL SOLUBLE SOLIDS, SUGARS, PUNGENCY AND FLAVOUR POTENTIAL IN IRRADIATED AND NONIRRADIATED RABI ONIONS DURING COMMERCIAL STORAGE

Treatment	Storage period (days)	Dry matter (%)	Total soluble solids (%)	Reducing sugars (%)	DPDS peak area/ (cm^{-2})	Pyruvic acid (moles/g)*
Control	Initial	13.96	11.50	5.24	4.20	9.58
Irradiated	,,	13.83	11.50	5.62	5.80	9.50
		Model Store				
Control	37	14.20	12.00	4.75	5.62	7.42
Irradiated	37	14.90	12.50	5.28	7.13	7.42
Control	69	13.40	11.50	4.35	4.55	8.10
Irradiated	69	13.45	11.25	4.86	5.06	6.78
Control	105	13.40	11.25	3.77	7.37	8.62
Irradiated	105	13.45	10.75	3.21	7.20	7.18
Control	132	14.10	11.50	4.81	4.91	10.34
Irradiated	132	14.75	12.75	5.22	6.39	12.02
		Chawl				
Control	37	13.85	11.50	4.75	4.74	7.70
Irradiated	37	13.75	12.50	5.28	5.63	6.94
Control	69	13.10	11.50	4.81	6.71	8.62
Irradiated	69	13.65	11.75	4.86	8.81	7.70
Control	105	13.25	10.50	3.99	6.89	7.42
Irradiated	105	13.75	11.25	3.73	5.22	7.42
Control	132	14.10	11.50	4.81	3.78	9.70
Irradiated	132	14.75	12.75	5.22	1.92	9.10

*Fresh tissue

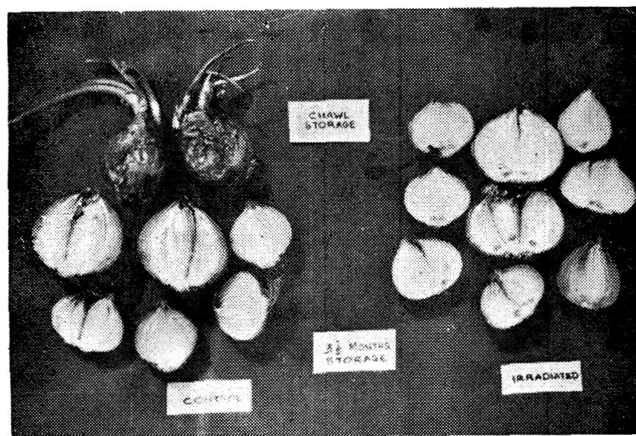

Fig. 2. Internal discolouration in irradiated onions due to dead meristem tissues or internal sprouts. Similar discolouration was seen in *chawl* and model store.

Discussion

The present study has shown that in rabi onions, under commercial storage, the losses due to microbial spoilage in *chawl* storage can be significantly reduced by improving the aeration through the onion pile as typified in the model store. The high rate of rotting in the *chawl* can be attributed to the build up of respiratory heat and humidity within the onion pile especially during the rainy months, creating conditions favourable for the proliferation of the spoilage pathogens. Storage losses to the extent of 40 to 50 per cent have been reported in onions stored in mud or straw cottages in Sudan after 4 or 5 months[11].

The influence of the main environmental factors which affect storage losses due to various causes is reflected in the results of the Rabi and Rangda crops. High humidities and ambient temperatures (25 to 32°C,

RH 60 to 80 per cent) existing during the rabi onion storage (June-October) would be expected to increase losses from rotting whereas the low humidities and temperatures (18 to 23°C, RH 25 to 60 per cent) existing during the Rangda onion storage (January to April) might increase desiccation losses but decrease those from rotting. The higher desiccation losses in rabi onions can be due to a higher rate of sprouting and rotting. An increased rate of desiccation can occur in sprouted bulbs due to a larger surface area and in rotted bulbs due to loss of textural integrity. The observation that despite the very low rotting, the losses due to desiccation in both rabi and Rangda onion stored in the model store was practically the same as in the chawl may be explained on the basis of the higher rate of air flow through the onion piles in the model store. Low temperatures are reported to shorten the dormancy and result in a more vigorous growth of the sprout under high humidity conditions[1,2]. However, the lower sprouting losses in Rangda onions especially in the model store is attributed to the extremely low relative humidity prevailing in winter and also to the increased ventilation rates which result in the drying up of the sprouts as they emerge. In contrast, the poor ventilation in the *chawls* can result in the build up of humidity pockets within the onion pile which help to promote sprouting and also sustain the continued growth of these sprouts.

Our results have shown the effectiveness of gamma irradiation for sprout inhibition of onions under commercial storage and when combined with sound storage practices, losses due to sprouting and desiccation can be reduced, particularly in rabi onions. Thus under the environmental conditions existing in Pimpalgaon, storage in a well aerated structure, as typified by the model store of NAFED, about 20 per cent more of the initial quantity of stored rabi onions could be saved by irradiation as compared to nonirradiated onions after a storage period of 4.5 months. Although irradiation did not show any beneficial effect on onions stored in the *chawl*, it is reasonable to expect results similar to that obtained in the model store by modifying the design of these *chawls* to improve aeration through all sides of the onion pile.

A preliminary techno-economic feasibility analyses of onion irradiation in a selected location in Nasik Dt. has indicated the economic viability of the irradiation process[7]. Further scaled up experiments under differing environmental conditions existing in major commercial storages are warranted to establish the precise benefits in terms of economic returns and also to test the market performance and consumer acceptance before the process can be applied on a commercial scale.

Acknowledgement

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Orotic Acid in Milk and Milk Products

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The orotic acid content in the milk of various breeds of cow and other species was in the order: 'Jersey' (52.60 $\pm$ 22.0 μg/ml) > 'Kankrej' $\times$ 'Jersey' > 'Kankrej' $\times$ 'Holstein' > 'Kankrej' > buffalo (19.55 $\pm$ 8.05 μg/ml) > goat > human milk (1.49 μg/ml). First lactation milk of 'Jersey' cows contained significantly higher ($P < 0.05$) orotic acid than the animals in second and third lactation but such relationship was not found in case of other breeds of cows and buffaloes. Orotic acid was also significantly higher in the milk of 'Jersey' cows in late lactation stage but not in case of 'Kankrej' cows and buffaloes. The order of orotic acid content in commercial dairy products was skim milk powder (489.43 $\pm$ 4.73 μg/g solids) > sweetened condensed milk > ice-cream > baby foods > processed cheese > *dahi* (55.53 $\pm$ 33.64 μg/g solids).

Orotic acid in the milk of most ruminant species namely bovine, ovine and caprine is an intermediate in pyrimidine biosynthesis[1]. It is synthesized in the cytoplasm from NH_3, CO_2 and aspartate through four enzymatic reactions[2]. Orotic acid reacts with 5-phosphoribosyl-l-pyrophosphate to form uridine-5′-monophosphate (UMP), the precursor of all other pyrimidine nucleotides. The absence of two enzymes viz. orotate phosphoribosyl transferase and orotidine-5′-monophosphate decarboxylase which are responsible for the conversion of orotic acid to UMP leads to the accumulation of orotic acid in the tissue cells resulting in a condition known as hereditary orotic aciduria[3]. There are two possible theories about the origin of orotic acid in milk. Firstly, it may be a part of blood orotic acid derived through blood flow in the mammary gland and secondly, it may be synthesized in the mammary gland tissues and diffused into milk[2]. Since its discovery[4] in the year 1904 its role in human physiology has not been clearly defined. Studies have shown that fatty liver is induced when rats were fed diets with high orotic acid content[5]. Bovine milk is the major source of orotic acid in human diet wherein its content[6] varies from 10 to 100 μg/ml and in exceptional cases it may go upto 800 μg/ml[7]. Analysis of a limited number of milk samples from rabit, pig, camel and women showed less than 1 μg/ml of orotic acid[8]. So far no report is available on the orotic acid content in the milk of Indian cows and buffaloes and the products made therefrom. In this investigation an attempt has been made to assess the content and variation of orotic acid in milk and milk products.

Materials and Methods

Throughout this study fresh milk samples of 'Kankrej', 'Jersey', 'Kankrej' $\times$ 'Jersey', 'Kankrej' $\times$ 'Holstein' breeds of cows and 'Surti' buffaloes were collected in the evening from individual animals of the herds maintained on the campus of this university. A few samples of buffalo and goat milk were also collected from the nearby villages.

After parturition changes in the orotic acid level from colostrum to normal milk were studied in two 'Kankrej' cows and two 'Surti' buffaloes. For this purpose samples were collected immediately after parturition (first colostrum) and till the subsequent secretions showed negative clot-on-boiling test. Orotic acid content was also assessed in some commercial dairy products.

Orotic acid in milk and milk products was determined by the method of Motz[9]. Standard deviation and correlations were calculated as per standard methods[10].

Results and Discussion

The orotic acid content (Table 1) in the milk of various breeds of cow is in the order: 'Jersey' > 'Kankrej' $\times$ 'Jersey' > 'Kankrej' $\times$ 'Holstein' > 'Kankrej'. The results suggest that cross breeding of exotic breed ('Jersey') with the indigenous breed of cattle ('Kankrej') brings down the orotic acid content in milk. The average orotic acid (μg/ml) in the milk of 'Jersey' cow is 52.6 and in those of 'Kankrej' cattle it is 28.0 but in the milk from their crosses is 30.7 μg/ml. Though milk from pure 'Holstein' cows was not available for comparison, it is reported[7] to be 81.1 μg/ml and the

TABLE 1. OROTIC ACID CONTENT IN MILK OF VARIOUS SPECIES

Species	No. of samples analysed	Mean$\pm$S.D. (μg/ml)
Cow		
Jersey	70	52.60$\pm$22.00
Kankrej x Jersey	6	30.73$\pm$ 8.16
Kankrej x Holstein	4	28.96$\pm$ 3.41
Kankrej	37	28.07$\pm$10.13
Buffaloe	30	19.55$\pm$ 8.05
Goat	20	12.14$\pm$ 4.98
Human	1	1.49 —

present study indicated a value of 28.96 μg/ml in Kankrej $\times$Holstein milk. These observations therefore suggest inheritance of breed differences. It is known that breed influences the orotic acid; 'Guernsey' and 'Jersey' cows produce milk with less orotic acid than do the 'Holstein', 'Brown Swiss' and 'Ayrshire'[7]. Individual variation of orotic acid in milk is large. Two of the 'Holstein' cows consistently produced milk containing upto 350 and 800 μg/ml. In this context it is necessary that orotic acid content is assessed in other breeds of Indian cattle extensively.

In buffaloe milk the orotic acid content is 19.55 μg/ml. Earlier report on analysis of a lone sample of water buffaloe[8] gave 29.0 μg/ml of orotic acid. In the milk of local breeds of goat, orotic acid content is 12.14 μg/ml and is almost the same as in some of the foreign breeds[8]. Lowest orotic acid was found in a lone sample of human milk (1.49 μg/ml) and it is known that human milk[8] contains the least amount of orotic acid.

Analysis of the data in relation to orotic acid content and its correlation with number of lactation in 'Kankrej' cows and 'Surti' buffaloes showed no significance. However the milk of 'Jersey' cows in the first lactation contained significantly ($P<0.05$) higher (57.77 μg/ml) orotic acid than those in the second (53.1 μg/ml) and the third (42.15 μg/ml) lactations. This observation is in conformity with other workers[7] who also observed less orotic acid in subsequent lactations in exotic breeds.

Grouping of animals as per the stage of lactation (early, mid and late) periods, orotic acid content showed no significant variation in the milk of 'Kankrej' cows and buffaloes but in the milk of 'Jersey' cows orotic acid content was significantly ($P<0.05$) higher during late lactation than during the early lactation period. This observation deviates from those of Jesse *et al.*[7], who observed that in 'Holstein' cows the orotate concentration reached a plateau by tenth week and remained constant (76.0 μg/ml) until near the end of lactation. The reason for such differences could be that in the present study, the animals were grouped as per early (1-100 days), mid (101-200 days) and late lactation (201-300 days) to scan the differences in orotic acid content but in the study by Jesse *et al.*[7] eight 'Holstein' animals were monitored throughout lactation period. Therefore, the individual differences might have masked the effect of stage of lactation.

To assess the status of orotic acid after parturition until secretion of normal milk, 2 'Kankrej' cows and 2 'Surti' buffaloes were selected. The secretions obtained immediately after parturition (first colostrum) and then after every 24 hr until the samples showed negative clot-on-boiling test were analysed for their orotic acid content. The orotic acid content in the milk of individual animals of the respective species showed identical trend and the pooled data are presented in Fig 1. It is observed that irrespective of the species, first secretion contained lowest orotic acid which gradually increased until normal milk was secreted. These observations are in accordance with those on 'Holstein' cows[7]. The results also reveal that the post-partum secretions from buffaloes contained considerably lower level of orotic acid than those from cows.

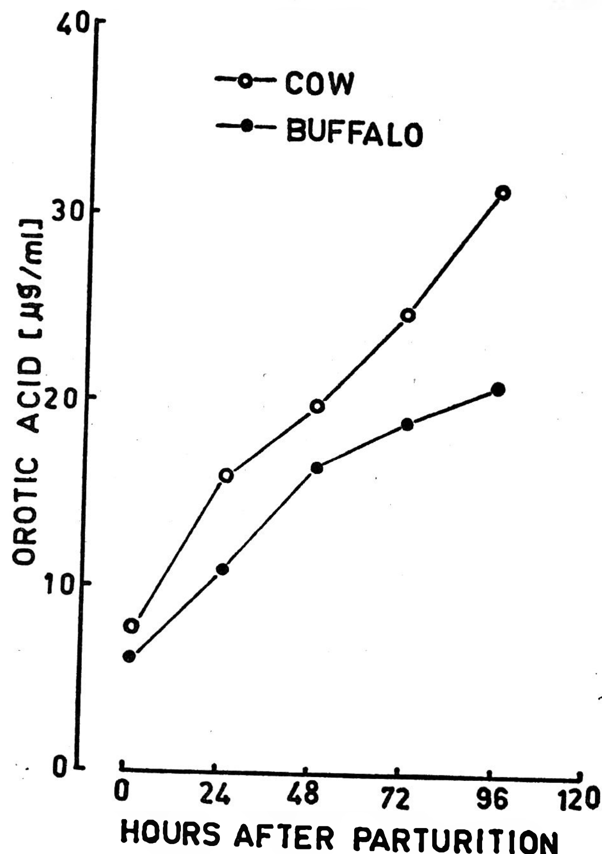

Fig. 1. Orotic acid content in post-partum secretions.

TABLE 2. OROTIC ACID CONTENT IN SOME MILK PRODUCTS

Product	No. of samples analysed	Range (μg/g)
Skim milk powder	2	486.08—492.78
Sweetened condensed milk	4	152.76—221.27
Ice-cream	3	151.55—177.40
Baby-food	3	113.55—173.49
Cheese	3	105.15—123.06
Dahi (Skim milk)	2	32.17— 78.90

The orotic acid content in commercial dairy products varied in the order: skim milk powder > sweetened condensed milk > ice-cream > baby foods > processed cheese > skim milk *dahi* (Table 2). The orotic acid content in almost all the products analysed in this study are considerably lower than those reported by Larson and Hegarty[8] on a limited number of similar products in USA. The lower orotic acid in commercial dairy products in India can be explained on the basis that orotic acid content in the milk of Indian cattle namely the 'Kankrej' and the cross bred cows is lower than the foreign breeds; furthermore orotic acid content of buffalo milk which contributes the major bulk for manufacture of dairy products in the country is lower. Lower orotic acid content in fermented products like *dahi* and processed cheese is due to the reason that orotic acid is utilised readily by various bacteria commonly used in the fermentation of dairy products[6,11] and its concentration therefore would depend upon the extent of fermentation in a particular product. Lower orotic acid content in sweetened condensed milk, ice-cream and baby foods in comparison to skim milk powder is explainable on the basis that these products contain fat and additives like sugar which add to the solids content and thereby reduce the orotic acid per unit weight of solids in these products.

The results of this study in general suggest that orotic acid content in the milk of 'Kankrej' cows, 'Surti' buffaloes and the cross bred cattle is significantly lower than the milk of 'Jersey and 'Holstein' cows and accordingly the commercial dairy products in the country also contain lower levels of orotic acid than similar products in the Western countries. If, orotic acid has anything to do with human health[2], the milk and milk products in India appear to be safer than the products in Western countries. However, to make these observations more realistic, it is necessary that milk of various breeds of cows and buffaloes in the country is examined for orotic acid content.

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Effect of Modified Atmospheres on the Shelf-Life of Pork Chops

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The effect of different atmospheres on pH, colour (Hunter's L, a, b values), smell and total bacterial counts on pork chops stored at 2°C was studied. The atmospheres used were: 1. air filled (control), 2. vacuum (95%), 3. high vacuum (99.9%), 4. carbon dioxide (99.9%). 5. nitrogen (99.9%) and 6. dinitrogen oxide (N_2O 99.9%). There were no remarkable changes in pH of fresh pork during storage regardless of atmospheric environment. The redness of chops decreased except in high vacuum. The chops had acceptable smell upto 9th day stored in air, N_2 and N_2O, upto 13th day in vacuum and high vacuum packed samples and even upto 16th day for CO_2 enriched packs. N_2 or N_2O did not inhibit the rate of bacterial growth.

The system of packaging in modified atmospheres or in vacuum to prolong the shelf-life of fresh meat has been investigated by several workers[1-6]. Chilled carcasses of beef were shipped as early as in 1938[7] using elevated levels of CO_2. Seiderman *et al.*[4] reported that a modified atmosphere containing 20 per cent CO_2 and 80 per cent N_2 retains natural colour of pork. Hermansen[8] found that vacuum packed pork samples became unacceptable after 12-13 days as compared to aerobically packed samples at 7-8 days of storage. Silliker and Wolfe[9] have studied the safety aspects related to storage of muscle foods in CO_2 enriched atmospheres. The present investigation was undertaken to study the effect of packaging in vacuum and in modified atmospheres such as CO_2, nitrogen and N_2O on pH, colour, microbiological and organoleptic changes in fresh pork chops stored at refrigeration temperature.

Materials and Methods

Fresh pork chops obtained from a meat processing factory were packaged in 42 laminated plastic bags made of PETP/X/PE/X, 12/5/70/5 with low oxygen permeability. Seven bags were assigned for each treatment— 1. air filled (control), 2. vacuum (95%), 3. high vacuum (99.9%), 4. CO_2 (99.9%), 5. nitrogen (99.9%) and 6. dinitrogen oxide (nitrous oxide, N_2O, 99.9%) and sealed with the help of Webomatic vacuum packaging machine. In treatments with modified atmospheres, air was completely removed by vacuum process and then flushed with the gases. The packaged chops were stored at 2°C in a refrigerator and the samples evaluated on 2, 6, 9, 13 and 16th day of storage for aerobic bacterial counts, odour, colour and pH.

pH was recorded directly by piercing the chops with combination electrode of Knick digital pH meter. Colour was measured within one hour after opening the packages using Hunterlab Tristimulus colorimeter, model D 25 A 9. The smell (odour) of the pork was evaluated subjectively based on 0-10 scale, used by meat processors laboratory. Aerobic total bacterial counts were determined with plate count agar incubating the plates at 30°C for 72 hr. The average of duplicate plate counts was expressed as number of organisms/g.

Results and Discussion

Hunter's L, a, b values, a measure of colour units, varied widely among treatments (Table 1). Pork chops became lighter in colour (L values) with increase of storage period in air, vacuum, CO_2, N_2 and N_2O. Adams and Huffman[1] made similar observations on colour of pork chops by using reflectance measurements and colour panel scores. However, Huffman *et al.*[10] have found that beef samples stored in N_2 atmosphere had least desirable colour as per subjective evaluation. The intensity of redness ('*a*' values) remained more or less same for high vacuum processed samples whereas other treatments showed decrease in redness. Bohnsack and Hopke[11] have shown that reddening capacity of meat was maintained in high vacuum packaging. Development of colour darkening related to metmyoglobin formation on the tissue

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TABLE 1. EFFECT OF VARIOUS ATMOSPHERES ON THE COLOUR OF FRESH PORK

Atmosphere	Hunter values for the indicated storage time (days)														
	2			6			9			13			16		
	L	a	b	L	a	b	L	a	b	L	a	b	L	a	b
Air	43.7	7.9	8.3	46.5	4.9	8.8	46.9	6.0	8.3	47.9	5.3	7.8	47.8	4.9	8.5
Vacuum	52.6	8.3	10.9	52.7	6.1	10.2	54.1	5.4	9.7	53.9	5.8	9.1	56.4	6.7	9.7
High vacuum	44.8	6.5	7.1	44.3	5.5	6.7	42.4	6.2	8.0	46.1	6.1	8.4	44.1	6.2	8.2
CO_2	52.6	5.8	9.3	55.2	1.9	8.3	52.2	3.9	9.1	54.9	2.9	8.8	55.8	3.5	9.1
N_2	46.4	7.7	9.7	50.9	4.2	6.9	49.4	4.8	7.7	49.2	5.6	8.4	39.7	8.2	6.2
N_2O	40.8	8.2	9.2	40.9	4.6	6.2	47.9	6.2	8.1	40.1	5.7	6.9	42.9	6.2	7.9

N.B.: 'L' measures lightness and values from 100 for perfect white to zero for black.
'a' indicates degree of redness when positive, grey when zero and greenness when minus.
'b' shows yellowness when plus, grey when zero and blueness when minus.

surface is the main disadvantage in the use of high CO_2 atmospheres in the fresh meat storage[12].

There were no appreciable changes in pH of pork chops during storage regardless of atmospheric environment (Table 2). However, Huffman *et al.*[10] reported that the changes in pH for all gaseous atmospheres (air, O_2, N_2 and CO_2 gas mixtures) follow the typical pattern of stored fresh meat, increasing gradually from pH 5.5 to 5.8 after 27 days storage at 1.1°C for beef steaks.

Chops stored in CO_2 showed drastically low counts throughout the storage period as compared to other treatments. These findings were in agreement with what can be accomplished with CO_2 enriched atmospheres[3,5,6]. N_2 and N_2O did not inhibit the rate of bacterial growth and even showed higher counts as compared to air packed samples and the results were consistent with the findings of Huffman[2], and Anjaneyulu and Smidt[13]. There were significantly higher counts of organisms in vacuum and high vacuum packaged samples compared to chops stored in CO_2. Christopher *et al.*[14] have reported that psychrotrophic bacterial counts of lean and fat surfaces of loins stored in 40 per cent CO_2+60 per cent N_2 were frequently significantly lower than counts of comparable sides of vacuum packed loins.

Pork chops had acceptable smell upto 9th day stored in air, N_2 and N_2O, upto 13th day for vacuum and high vacuum packed chops and even upto 16th day for CO_2 enriched packs. These findings were consistent

TABLE 2. CHANGES IN pH, SMELL AND BACTERIAL COUNTS OF FRESH PORK CHOPS STORED AT 2°C IN DIFFERENT ATMOSPHERES

Atmosphere	Storage time (days)												
	2		6			9			13			16	
	pH	Smell	pH	Smell	Bact. count	pH	Smell	Bact. count	pH	Smell	Bact. count	pH	Smell
Air	5.43	VG	5.37	G	4.5×10^3	5.44	N	8.0×10^4	5.48	VLB	1.4×10^6	5.46	VB
Vacuum	5.40	VG	5.33	G	5.5×10^3	5.35	N	3.8×10^4	5.57	N	4.8×10^5	5.50	B
High vacuum	5.46	VG	5.48	G	3.2×10^3	5.44	N	3.6×10^4	5.49	N	1.0×10^6	5.50	LB
CO_2	5.44	VG	5.45	G	1.2×10^3	5.44	N	2.7×10^3	5.54	N	2.6×10^4	5.48	N
N_2	5.49	VG	5.49	G	9.8×10^3	5.45	N	1.2×10^5	5.53	VLB	8.1×10^6	5.47	VB
N_2O	5.48	VG	5.50	G	6.1×10^3	5.42	N	7.1×10^4	5.62	VLB	1.5×10^6	5.55	B

VG: Very good; G: Good; N: Neutral; VLB: Very little bad, V.B.: Very bad, B: Bad, LB: Little bad

with the results of Spahl *et al.*[6], Hermansen[8] and Silliker *et al.*[9] The present study indicated that CO_2 enriched atmosphere was suitable for packaging of fresh pork while vacuum packaging was observed to maintain fresh meat colour during storage. N_2O may not have the ability to extend the shelf-life of fresh pork.

Acknowledgement

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Comparison of Organoleptic and Related Characters of Buffaloe Meat with Beef

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Comparison between buffaloe meat and beef (*Longissimus dorsi* and *Semimembranosus* from the right side of the carcasses) collected from the live weight of 51 to 175 kg revealed that they were almost similar in: colour of lean, total myoglobin content, shear values, muscle fibre diameter, texture, juiciness and flavour. Though buffaloe muscles recorded lower ultimate pH and lower water holding capacity, significant differences were observed in the (101 to 125 kg) and (126 to 150 kg) weight groups only.

There is a great potential for meat production from buffaloes in India which constitute about half the total number of world cattle population. Preliminary studies conducted elsewhere revealed that buffaloe meat is comparable to beef in eating quality[1-3], but, adequate information is not available on the quality of meat

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from buffaloes at different stages of maturity groups to serve as fresh meat or to incorporate in various meat products. Hence, the present investigation was undertaken to make a comparative study of the organoleptic characters and related objective evaluation of buffaloe meat with beef from cattle at different live weight groups.

Materials and Methods

Twenty five males, each of locally available buffaloes (*Bos bubalis*) and Zebu cattle (*Bos indicus*) were randomly selected in a manner to fit into five live weight groups ranging from 51 to 175 kg. The animals were electrically stunned and slaughtered at Bacon Factory, Gannavaram. After 24 hr of chilling in a cold storage, *Longissimus dorsi* muscle between 7th thoracic and 2nd lumbar vertebrae and *Semimembranosus* muscle from the right side of the carcasses were separated and stored in a refrigerator at 2–4°C for further analysis after 72 hr following slaughter.

Ultimate pH was recorded; for estimation of water holding capacity (WHC), filter paper press method of Grau and Hamm[4] was adopted with the following modifications. Meat sample (300 mg) was placed on a Whatman No. 1 filter paper between two glass plates and subjected to a pressure of 100 g for 3 min. Area of moisture absorption was read with a compensating polar planimeter and expressed in sq. cm. Three such impressions were obtained and the average was taken as expressible juice (EJ) of the sample. Increase in EJ was interpreted as decrease in WHC of the sample.

Total myoglobin content in the muscle was estimated according to the procedure of Fleming *et al.*[5]

Two cores, 1.25 cm in diameter and parallel to muscle fibres were obtained from samples frozen overnight in a deep freezer and were then cooked in refined groundnut oil at 80°C for 2 min. They were sheared through at three places in a Warner-Bratzler shear press and the mean was taken as shear values of the sample. A decrease in shear value was interpreted as increase in muscle tenderness. Muscle fibre diameter was calculated as per the procedure of Hiner *et al.*[6] using a compound microscope.

Steaks meant for subjective evaluation were scored for colour of lean by a 7-member semi-trained panel

TABLE 1. MEAN VALUES AND SCORES FOR DIFFERENT PARAMETERS STUDIED IN BEEF AND BUFFALOE MUSCLES

Wt. group (kg)	Source of muscle	Total myoglobin (mg/g tissue)	Ultimate pH	Expressible juice (cm^2)	Shear value (kg)	Muscle fibre diam. (μ)	Scores: Raw colour	Tenderness	Texture	Juiciness	Flavour
				Longissimus dorsi **muscle**							
51–75 (I)	Beef	1.03	5.53	3.47	2.41	30.24	2.50	4.74	4.66	4.18	4.50
	Buffaloe	1.19	5.62	4.39	2.85	32.41	3.40	3.58	3.88	3.48	3.66
76–100 (II)	Beef	1.16	5.68	3.74	3.26	37.03	3.20	4.34	4.68	4.42	4.88
	Buffaloe	1.20	5.71	3.42	3.65	35.96	3.44	3.80	4.08	3.94	4.84
101–125 (III)	Beef	1.36	5.50	3.38	3.83	41.46	3.88	3.94	4.24	3.70	5.12
	Buffaloe	1.30	5.56	4.06	3.76	36.89	3.46	3.60	3.86	3.74	4.44
126–150 (IV)	Beef	1.34	5.94	2.90	4.35	42.84	4.38	3.24	3.22	3.56	4.28
	Buffaloe	1.36	5.56	4.43	4.27	38.65	3.62	3.58	3.80	3.50	4.04
151–175 (V)	Beef	1.61	5.65	4.04	4.96	48.53	5.00	2.98	2.68	2.98	4.42
	Buffaloe	1.32	5.63	4.30	4.46	48.00	5.70	3.18	3.06	3.32	4.36
				Semimembranosus **muscle**							
51–75 (I)	Beef	1.22	5.67	4.05	2.67	28.31	2.50	4.48	4.52	3.92	4.56
	Buffaloe	1.25	5.44	3.86	3.79	31.00	3.50	3.24	4.16	4.04	3.30
76–100 (II)	Beef	1.22	5.60	4.21	3.43	35.43	3.40	4.20	4.20	4.08	3.88
	Buffaloe	1.31	5.42	4.54	3.74	37.83	3.60	3.20	3.74	3.84	4.44
101–125 (III)	Beef	1.74	5.46	4.19	4.08	39.99	3.82	3.56	3.88	3.68	4.96
	Buffaloe	1.33	5.51	4.98	4.58	36.12	3.50	3.62	3.80	3.62	4.82
126–150 (IV)	Beef	2.25	5.62	3.92	5.09	43.19	4.34	3.06	2.82	3.10	4.26
	Buffaloe	1.76	5.43	4.28	4.24	37.55	3.84	2.96	3.22	3.12	4.20
151–175 (V)	Beef	2.37	5.60	5.05	5.17	46.23	4.72	2.36	2.48	2.60	3.80
	Buffaloe	2.48	5.62	4.63	4.69	46.11	4.74	2.96	3.16	3.06	4.22

using a scale of 1 (very light) to 7 (very dark). Steaks were roasted at 180°C until an internal temperature of 70°C was reached. After cooling to room temperature, the panel evaluated them for tenderness, texture, juiciness and flavour on a 7-point Hedonic scale.

Means, standard deviations and analysis of variance of the parameters studied were calculated[7] and presented in Tables 1 and 2. In the parameters, where significant differences were observed between beef and buffaloe muscles, further analysis (Table 3) was attempted to study differences in each weight group.

Results and Discussion

No significant differences between species in colour of lean were observed in this study (Table 2). Ogan-janovic[2] and Wilson[3] reported that buffaloe muscles were slightly darker than the beef of cattle. Joksimovic[8] suggested that the lower fat content of buffaloe meat could be the cause of the darker colour and postulated that this could be changed by improved nutrition and breeding. Since, both zebu cattle and buffaloes slaughtered in this study were of lean type, raised under grazing only, differences in colour of lean due to intramuscular fat content were not expected. Total myoglobin levels which determine colour of lean were almost in the same ranges as no significant differences were observed between the muscles of the two species.

A significant difference was observed in the ultimate pH between the two species (Table 2). Beef muscles recorded higher pH values. But the differences in ultimate pH between beef and buffaloe muscles were significant only in the IV weight group (Table 3). Lawrie[9] predicted that the complement of enzymes which effect glycolysis might vary among individual animals, breeds and species. The possibility of such a variation occurring between the buffaloes and cattle involved in this study cannot be ruled out. Arambulo[10] and Wilhelms[11] also reported that buffaloe meat recorded a low ultimate pH.

Highly significant differences were also observed in water holding capacity between the two species. Beef muscles recorded lower expressible juice than those of buffaloes indicating that they were superior in WHC. But the differences between the two species were significant only in the III and IV weight groups (Table 3). This observation might be of some interest since in the IV group, a low ultimate pH was also reached and this factor was reportedly associated with decrease in WHC[4]. Since these observations were noticed in one or two weight groups only, further studies involving a larger sample of animals is desirable regarding differences in ultimate pH and water holding capacity in beef and buffaloe muscles.

TABLE 2. MEAN VALUES, STANDARD DEVIATIONS AND F VALUES OF PARAMETERS STUDIED IN BEEF AND BUFFALOE MUSCLES

Parameters	Beef muscles Mean±S.D.	Buffaloe muscles Mean±S.D.	F value
Total myoglobin (mg/g of wet tissue)	1.53±0.44	1.45±0.38	3.114
Ultimate pH	5.63±0.13	5.55±0.09	5.957*
Expressible juice (cm²)	3.90±0.55	4.29±0.41	11.097**
Shear value (kg)	3.93±0.94	4.00±0.53	0.433
Muscle fibre diam (μ)	39.33±6.24	38.05±5.06	1.829
Raw colour score	3.77±0.83	3.88±0.71	1.191
Tenderness score	3.69±0.73	3.37±0.28	5.508*
Texture score	3.74±0.82	3.68±0.37	0.231
Juiciness score	3.62±0.55	3.57±0.32	0.243
Flavour score	4.47±0.42	4.23±0.45	3.169

* $P<0.05$ ** $P<0.01$

TABLE 3. COMPARISON OF PARAMETERS WITH SIGNIFICANT DIFFERENCES BETWEEN BEEF AND BUFFALOE MUSCLES IN DIFFERENT LIVE WEIGHT GROUPS

Wt. groups (kg)	Ultimate pH			Expressible juice (sq.cm)			Tenderness scores		
	Beef	Buffaloe	F value	Beef	Buffaloe	F values	Beef	Buffaloe	F value
(I) 51–75	5.60	5.53	1.087	3.76	4.13	1.877	4.61	3.41	15.686**
(II) 76–100	5.64	5.57	1.087	3.98	3.98	0.001	4.27	3.50	6.460*
(III) 101–125	5.48	5.54	0.652	3.79	4.52	7.739**	3.75	3.61	0.214
(IV) 126–150	5.78	5.50	17.913**	3.41	4.36	12.685**	3.15	3.27	0.157
(V) 151–175	5.13	5.63	0.009	4.55	4.47	0.086	2.67	3.07	1.743

*$P<0.05$ **$P<0.01$

Though the taste panel scored beef samples to be more tender in the I and II weight groups, shear values did not indicate the presence of significant differences between the two species. Matsukawa *et al.*[12] also reported non-significant breed differences in shear force values in Bos (Red 'Sindhi' and 'Friesian') and Bubalis ('Murrah') meat. Beef and buffaloe muscles were almost similar in muscle fibre diameter, texture, juiciness and flavour.

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Localisation of Significant Constituents of Turmeric

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The localisation of curcumin, essential oil, starch and polyphenols in turmeric was studied with respect to maturation and sprouting. Sodium carbonate solution and boric acid reagent used for colorimetric determination were used for histochemical studies of curcumin. Curcumin and essential oil were found to be distributed throughout the rhizome surrounded by hyaline cells. Starch was stored in the inner core of the rhizome. Polyphenols were located in the scale leaves, vascular bundles and growing tips of axillary buds of the rhizomes.

Turmeric (*Curcuma longa* Linn) is an important spice valued for the characteristic yellow colour and flavour. It has 1.8 to 5.4 per cent of curcumin, the pigment and 2.5 to 7.2 per cent of essential oil[1]. The spice is also relatively rich in starch especially at the mature stage. The location and distribution of the different constituents have not so far been studied. While histochemistry has been well utilized for locating chemical constituents in animal products[2], very few reports of such studies are available with regard to products of plant origin. Recently histochemistry has been used to locate the phenolic substrates responsible for blackening in pepper[3]. The present investigation was aimed at histochemically locating the significant constituents of turmeric rhizome as well as their development during maturation.

Materials and Methods

Turmeric at different stages of growth like young (5 to 6 months), mature (8 to 9 months) and sprouting rhizomes was collected from a local garden.

Sections of 30 μ thickness were prepared with a microtome; for fresh material, a freezing microtome was used. They were treated by dipping in each of the specific reagents for about 20 to 30 sec. Different sections were used for different reagents.

Aqueous solution of 10 per cent sodium carbonate and 10 per cent aqueous boric acid-concentrated sulphuric acid-glacial acetic acid mixture (1:1:1) were used for locating curcumin. Saturated solutions of Sudan III and Sudan IV in ethylene glycol were used for staining the essential oil cells; 0.3 per cent solution of iodine in 6 per cent potassium iodide[4] was used for locating starch.

Phenolic reagent, bis-diazotised benzidine[5] was prepared by mixing 3 parts of benzidine solution in hydrochloric acid and 2 parts of 10 per cent aqueous sodium nitrite solution. Vanillin-hydrochloric acid reagent was prepared by adding 1 part concentrated hydrochloric acid to 2 parts of 10 per cent alcoholic vanillin[5,6].

Photomicrographs were obtained with a PZO Warzawa biological microscope, MB 10. Scanning electron micrographs were taken with a Jeol 80 model scanning electron microscope after the sections were sputtered with gold ions.

Results and Discussion

Colour change of curcumin with alkali has been recognized.[7] Boric acid-sulphuric acid-acetic acid mixture have already been used as reagents for colorimetric determination of curcumin[8]. These were successfully used in appropriate concentrations as histochemical reagents for locating curcumin in turmeric sections, sodium carbonate giving red colour and boric acid giving crimson.

Differentiation of curcumin cells took place in the very young rhizomes as indicated by colour reaction. Curcumin was found to be localised in isolated circular pigment cells, situated among a group of hyaline cells, (Fig. 1). No connection between vascular supply and these pigment cells was noticed and curcumin filled the entire cell cavity (Fig. 2). The pigment from these cells showed the same R_f as that of curcumin on chromatoplates (silica gel G; benzene-methanol (80:6) solvent system). When viewed under UV light using fluorescence microscope, pigment cells displayed yellowish green fluorescence characteristic of isolated curcumin. In the young rhizomes, the curcumin cells were found to be located more towards the outer regions of the rhizome whereas at mature stage, the number of curcumin cells was relatively larger in the inner regions of the rhizome.

Apart from the curcumin cells, certain cells filled with lightly coloured viscous droplets were also noticed under the microscope at all stages. Section of a mature rhizome is presented in Fig. 3. When treated with Sudan III or Sudan IV dissolved in ethylene glycol, these cells alone were coloured red indicating that they were essential oil cells (Fig. 1). They were distributed uniformly in the outer and inner regions of the rhizome at mature stage. Just as the pigment cells, each oil cell was surrounded by four or five hyaline cells. It was noticed that an alcoholic solution of Sudan dye could not be used because of dissolution of curcumin and subsequent smearing which interfered with the microscopic examination. Neither the pigment cell nor the essential oil cell was noticed in roots of turmeric.

Since no specific stain could be standardised for essential oil, Sudan III and Sudan IV which are used for locating lipids, were used. The effectiveness of the stains was checked by isolated essential oil. Both Sudan III and Sudan IV in ethylene glycol stained essential oil cells in the rhizome effectively. The identity of the cells as essential oil bearing was also confirmed by optical microscopic and scanning electron microscopic studies, when sections were steam distilled for 30 min the cells identified as essential oil cells were found to be emptied. There appeared to be no significant deposition of other lipids, like triglycerides in the rhizome of all stages.

Starch grains were located by the blue stain produced with iodine solution. In young and sprouting stages, starch deposition was not seen. Accumulation of starch in the rhizomes started after the fifth month of growth. Starch was present in large amounts at mature stage stored in the parenchymatous cells of the pith (Fig. 4). Under optical microscope starch grains were found to be simple and oval in shape. Scanning electron microscopic study also showed the oval shape with hilum having a cap-like orientation (Fig. 5). Each starch grain was found to be a unit by itself loosely bound in the cavity of the cell in which it is stored, and deposited along the periphery of the inner cavity wall.

At young stage when the rhizomes were treated with bis-diazotised benzidine, the brown colour indicative of presence of polyphenols was developed on the scale leaves covering the rhizome, vascular bundles and the growing tip of axillary buds of the scale leaves (Fig. 6). In the case of root, the entire section consisting of one or two layers of periderm, meta and protoxylem vessels and parenchymatous pith cells of the root showed reactivity (Fig. 7).

At the mature stage, intensity of colour reaction was generally very little, thereby indicating low concentrations of phenols. Polyphenols were located in xylem vessels and xylem parenchyma. However, while sprouting, roots and sprouting shoots showed intense polyphenol reaction.

Pink colour formation with vanillin-hydrochloric acid, indicative of presence of flavanols was noticed in periderm of young rhizomes in the form of channels. Flavanols were absent in the inner regions of the rhi-

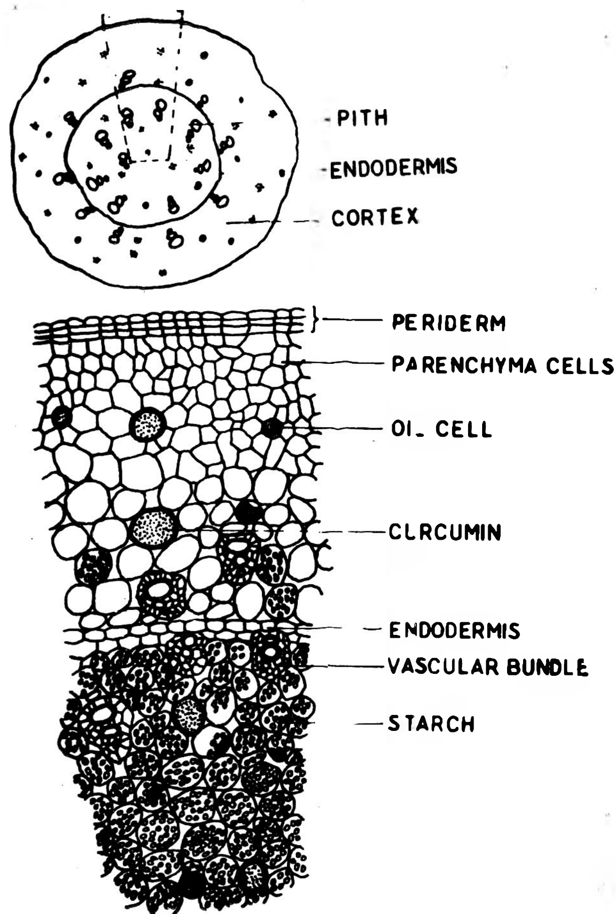

Fig. 1. Cross section of mature turmeric rhizome showing the distribution of curcumin cells, essential oil cells and starch.

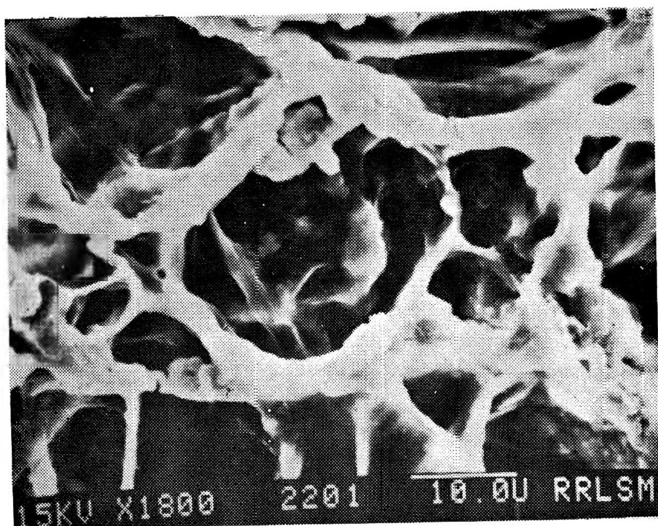

Fig. 2. Scanning electron micrograph of mature rhizome showing the curcumin cell (central prominent cell). Magnification: 15 KV×1800.

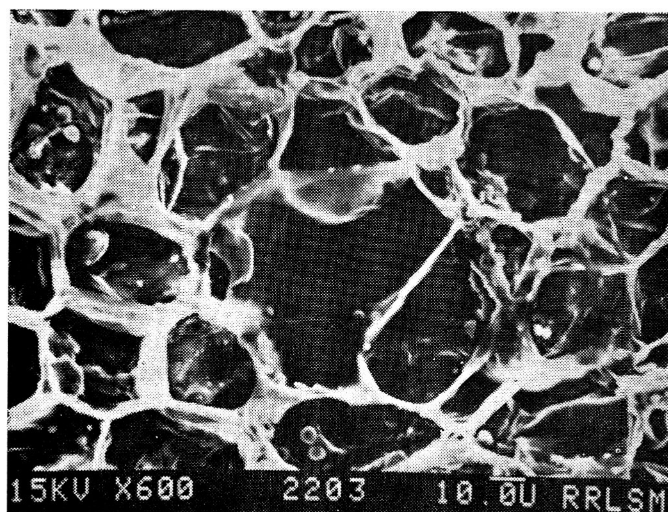

Fig. 3. Scanning electron micrograph of mature rhizome showing the essential oil cell (central large transparent cells). Magnification: 15 KV×600.

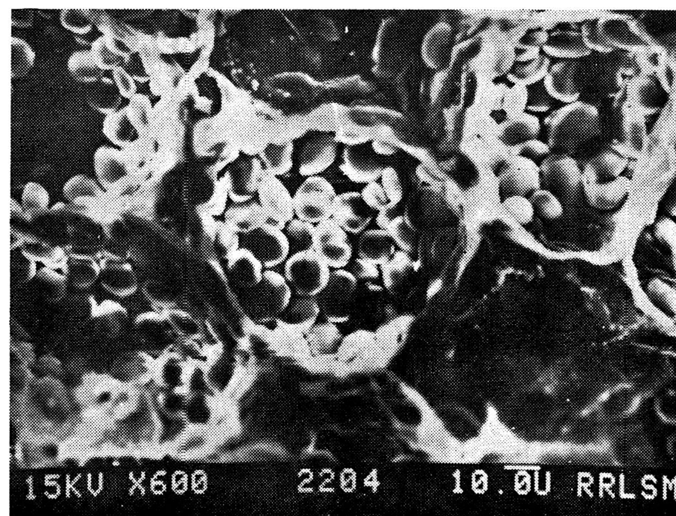

Fig. 4. Scanning electron micrograph of cross section of mature rhizome showing the deposition of starch grains in paranchymatous cells of the pith. Magnification: 15 KV×600.

zome even where phenolics were indicated by bis-diazotised benzidine reactivity. In the case of root, flavanol was seen in the cells outside the endodermis (Fig. 7). In sprouting shoots, flavanols were localised in the scale leaves and meristematic regions of the bud in round individual cells situated in the plerome alone (Fig. 8). In the periblem, flavanols weie absent. Because of the interference of curcumin with alkali, Folin Denis reagent[6] could not be used for locating phenolic constituents in turmeric.

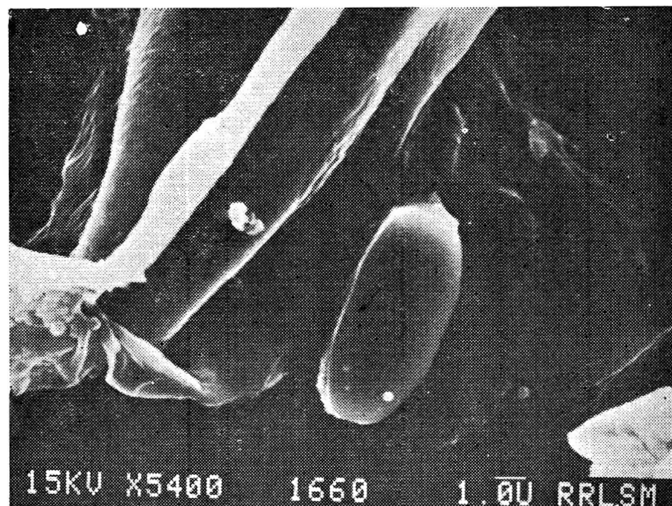

Fig. 5. Scanning electron micrograph of a single starch grain showing cap-like orientation at the top of the grain. Mangification: 15 KV×5400.

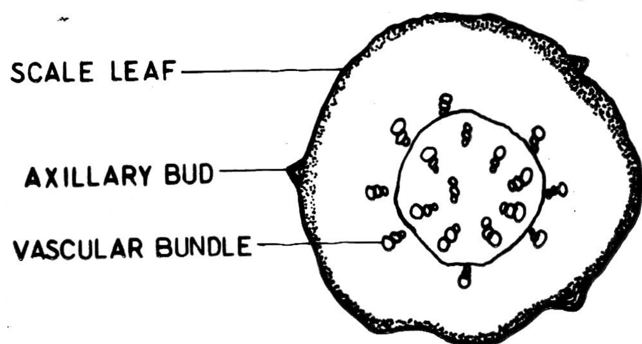

Fig. 6. Cross section of young rhizome showing bis-diazotised benzidine staining.

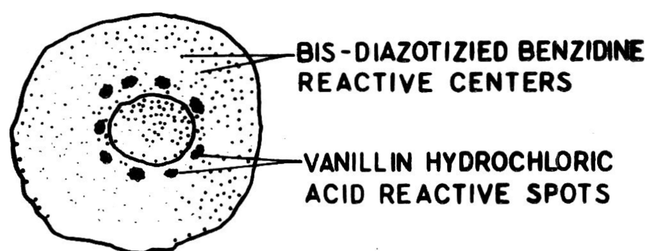

Fig. 7. Cross section of root in sprouting rhizome showing phenolic reactivity.

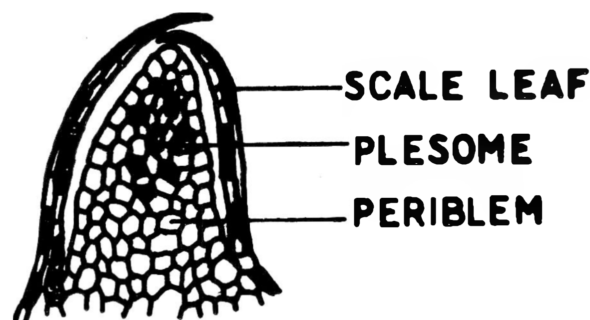

Fig 8. Growing tip (LS) of the sprouted turmeric with shaded portion indicating reaction with vanillin hydrochloric acid.

Acknowledgement

Authors thank Mr Peter Koshy for SEM studies and Prof. C. A. Ninan, Head of Department of Botany, University of Kerala for his keen interest in the work. One of the authors (C. K. Mangalakumari) is grateful to CSIR for fellowship.

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Dissipation Pattern of Monocrotophos and Quinalphos Residues from Sweet Orange Fruits

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The buildup of monocrotophos and quinalphos residues on sweet orange fruits following their application and progressive dissipation pattern was studied. Both the insecticides persisted beyond three weeks after spray. Monocrotophos was found accumulating in fruit pulp. Quinalphos also penetrated inside the pulp in limited amounts slowly. Residues dissipated at the half life of 6.62 to 8.90 days for monocrotophos and 7.32 to 9.23 days for quinalphos. Both the insecticides required more than 32-34 days to come down to the tolerance limits.

The pest complex in sweet orange throughout the cropping season from new flush to fruiting warrant the use of pest control chemicals for their effective control. Among a number of insecticides, monocrotophos (dimethyl (*E*)–1–methyl–2–methyl carbamoylvinyl phosphate) and quinalphos (*0-0*-diethyl *0*-quinaxalin-2-yl phosphorothioate) have been screened and recommended for use at their effective application rates.[1,2] However, continuous use of these chemicals through fruiting stage may result in the buildup of their residues on fruits which will adversely affect the consumers. It is all the more alarming because fruits are consumed fresh. Present studies were conducted to evaluate the persistence pattern of the residues and find out the safety waiting periods.

Materials and Methods

Sweet orange, *Citrus sinensis* Osbeck, 'Mosambi' variety at the fruiting stage in a three year old orchard of Indian Institute of Horticultural Research, Hesaraghatta, Bangalore was selected for the supervised field trial. The plants bearing fruits close to maturity were spray treated with monocrotophos and quinalphos each at 0.05 and 0.1 per cent spray concentrations with a high-volume sprayer at the rate of 2 l/tree. Three replications of each treatment concentration taking one tree (bearing about 50 fruits) as one replication, were laid out in a randomised block design experiment. Three fruits, comprising one gross sample from each of the replications, were drawn at random immediately after the spray treatment for determination of initial insecticide deposits and at periodic intervals of 3, 7, 10, 15 and 21 days after spraying for residue analysis.

Fruit samples were processed by peeling the fruits to separate fruit pulp from rind. The residues of monocrotophos and quinalphos from representative 25 g rind samples and 50g pulp samples were extracted by blending with methylene chloride (3×50 ml and $3\times$ 100ml respectively) in a Waring blender for 1.5 min each time. The extracts containing monocrotophos were processed through partitioning cleanup technique[3] for determination of residues through enzyme inhibition technique[4] using out-dated human blood plasma as the source of choline-esterase enzyme. The extracts containing quinalphos residues were concentrated in a Kuderna Danish evaporator and quantitatively transferred to hexane layer through solvent partitioning in a separatory funnel. The hexane extracts were concentrated to about 5 ml in a rotatory flash evaporator and residues were cleaned up from the other coextractives using column chromatography through the layers of neutral alumina grade III (5g) overlaid by a layer of florisil (5g) in 10mm$\times$300mm glass columns prewashed with 100 ml hexane. The cleaned up quinalphos residues were determined by gas liquid chromatography using Varian GC-3700 model equipped with thermionic specific detector and mounted with 5 per cent OV 101 on Gas Crom Q support of 100-120 mesh, S. S. 6mm$\times$50 cm column. The operational parameters were: column temperature 200°C, inlet temperature 210°C, detector temperature 230°C; gas

Contribution No. 34/85.

flow rates of 30, 4 and 140 ml/min for nitrogen, hydrogen and air respectively; Bias Voltage 4, bead current 6; attenuation 16×10^{-11} and chart speed 1 cm/min. Under above parameters quinalphos separated as a single peak at the retention time of 1.9 min.

The recovery tests from the fortified control samples of rind and pulp of the sweet orange fruit at known quantities of monocrotophos and quinalphos were carried out simultaneously to find out the efficiency of the analytical techniques followed for residue analysis. Average recoveries ranged in the order of 87 to 91.5 per cent for monocrotophos and 90.7 to 94.3 per cent for quinalphos. Comparatively higher recoveries were found from fruit pulp than from fruit rind, as the extracts from rind carried higher amounts of co-extractives. The residue data were subjected to statistical analysis[5] in order to work out half life ($t\frac{1}{2}$ value) and waiting periods (T_{tol} values) for both the insecticides at their respective application rates.

Results and Discussion

The spray schedules of monocrotophos and quinalphos insecticides each at 0.05 and 0.1 per cent field strength to sweet orange plants in their bearing stage resulted in the initial buildup of 2.16 and 5.46 ppm monocrotophos and 3.02 and 5.83 ppm quinalphos on rind of the fruit. Periodic analysis of the treated fruits over 3 weeks showed progressive dissipation of residues from either of the two chemicals. Simultaneously the residues were also found moving from their site of application to inner tissues of the pulp in the fruit depending on the extent of systemic activity of the chemical. As a result, monocrotophos being highly systemic was found making its way to pulp immediately after spray treatment as compared to quinalphos which has limited systemic activity (Table 1). The proportional increase of monocrotophos residues in pulp as compared to aging residues on rind reflect the continuous movement from surface to inner tissues along with faster rate of dislodging and dissipation of surface residues to that of free and bound residues inside the fruit pulp. The bonding of pesticide molecule with plant constituents to form glucosides, etc. result in longer persistence of residues. The systemic movement of insecticide residues also affected their dissipation pattern since the migrated residues escaped the effects of physical forces of pesticide decomposition. Therefore monocrotophos experienced comparative slow dissipation in initial stages as compared to quinalphos (Table 2), However, more than 81 to 96 per cent of the residues were found lost within three weeks of foliar spray. Monocrotophos residue dissipated at the rate of 8.98 and 6.62 days half life from the two treatment concentrations to require waiting periods of 32 to 34 days based on the tolerance limit of 0.2 ppm[6]. Quinalphos dissipated at the rate of 9.23 and 7.32 days half life to require 32 days waiting period based on its tolerance limit of 0.25 ppm for sweet orange fruits (Table 3).

Longer persistence of organophosphorus insecticides have been reported on citrus fruits due to slow decay of their residues through hydrolytic breakdown of organophosphorus esters[7]. Since these compounds are highly susceptible to alkaline hydrolysis[8], the acidic nature of sweet orange therefore did not allow the major pathway of their degradation. Further the surface residues may have been held up in oil sacs present in the rind of fruits and escaped the severity of physical forces of pesticide degradation.

TABLE 1. RESIDUES OF MONOCROTOPHOS AND QUINALPHOS ON SWEET ORANGE FRUITS

Days after treatment	Treatment (%) concn.	Average residues (ppm) Monocrotophos Rind	Monocrotophos Pulp	Quinalphos Rind	Quinalphos Pulp
0	0.05	2.16	0.22	3.02	N.D.
	0.10	5.46	0.32	5.83	N.D.
3	0.05	1.46	0.61	1.66	0.42
	0.10	3.40	0.83	2.91	0.51
7	0.05	1.07	0.82	1.15	0.50
	0.10	1.84	1.37	1.83	0.89
10	0.05	0.98	0.78	1.02	0.45
	0.10	1.25	1.16	1.27	0.68
15	0.05	0.46	0.47	0.67	0.36
	0.10	0.68	0.57	0.83	0.52
21	0.05	0.22	0.23	0.36	0.19
	0.10	0.32	0.31	0.47	0.33

TABLE 2. DISSIPATION PATTERN OF MONOCROTOPHOS AND QUINALPHOS FROM SWEET ORANGE FRUITS

Insecticides	Treatment (%) concn.	% loss of residues at indicated days 3	7	10	15	21
Monocrotophos	0.05	13.02	20.58	26.05	60.92	81.09
	0.10	26.81	44.46	58.30	78.37	89.10
Quinalphos	0.05	31.12	46.36	57.32	65.89	81.78
	0.10	41.33	53.34	66.55	76.84	96.27

TABLE 3. SAFETY EVALUATION CONSTANTS OF MONOCROTOPHOS AND QUINALPHOS FOR SWEET ORANGE FRUITS

Insecticides	Treatment concn (%)	Initial residues (mg/kg)	regression equation	Half life ($t_{\frac{1}{2}}$ in days)	Waiting period (T_{tol} in days)
Monocrotophos	0.05	2.38	Y=2.4535+0.0335×	8.98	34.40
	0.10	5.78	Y=2.7884+0.0457×	6.62	32.54
Quinalphos	0.05	3.02	Y=2.4621+0.0326×	9.23	32.64
	0.10	5.83	Y=2.7206+0.0411×	7.32	32.18

Acknowledgement

The authors wish to thank Dr. K. L. Chadha, Director of the Institute for his interest and support in the present study.

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NATIONAL CONFERENCE ON FOOD AND ALLIED INDUSTRIES

June 20th and 21st 1986

Organised by The Association of Food Scientists and Technologists (India), Calcutta Chapter, Jadavpur University, Calcutta-700 032 in collaboration with Small Industries Service Institute, Min. of Industry GOI, Calcutta & Jadavpur University, Calcutta.

Industries of cereals and pulses, fats and oils, fruits and vegetables, fish, meat and poultry, confectionery, beverages, spices and condiments, dairy products, snack foods, plant and machinery, packaging machine, waste disposal, abatement of pollution and quality control with special reference to legal aspects have been earmarked for discussion during the two days.

While time for reading research papers will not be there, arrangements will be made for poster presentation of research papers if intimation is received with advance registration by 15th May 1986. Each presentation will be limited to space of a maximum dimension of 2×2 metre.

It is also proposed to hold an Exhibition on machinery and processes pertaining to food and allied industries.

Venue of the Conference is:

The Auditorium of Indian Institute of Chemical Engineering, Jadavpur University Campus, Calcutta-700 032.

Prof. M. N. Chakraborty,
Chairman
Organising Committee

RESEARCH NOTES

BENZALDEHYDE AS A MEASURE OF BITTER KERNELS IN SWEET ALMONDS

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Methods for the determination of benzaldehyde in almonds are described. Concentration of benzaldehyde has been found to be related to the proportion of bitter kernels in sweet almonds.

Bitter almonds generally get admixed with sweet almonds and their detection poses serious problems. In the international trade, only 3 per cent of bitter kernels are allowed and at present, sensory evaluation is mainly used to determine the percentage of bitter kernels in sweet shelled almonds. Despite being extensively used, this method is laborious, time consuming and very costly.

Bitterness in almonds is due to the presence of cyanogenetic glycoside amygdalin (mandelonitrile gentiobioside)[1]. Besides being bitter, amygdalin is toxic as it releases hydrocyanic acid on hydrolysis by the native hydrolases and acids. Since each molecule of amygdalin besides releasing hydrocyanic acid and glucose also produces a molecule of benzaldehyde, its concentration may be related to the weight of bitter kernels. The present investigation was undertaken to determine the relationship between benzaldehyde concentration and bitter kernels.

Different grades of sweet almonds were obtained from the local market. Bitter almonds were obtained from Delhi. Benzaldehyde was procured from Glaxo India and methanol and ethanol used in the colorimetric estimation of benzaldehyde were made carbonyl free by refluxing with zinc dust and soidum hydroxide and subsequently distilling in an all glass apparatus.

Ten grams of almond powder was mixed with 50 ml distilled water and kept at room temperature. After 2 hr the mixture was treated with 5-10 ml methanol and the contents were steam distilled collecting 150 ml of the distillate in a 250 ml flask. The distillate flask was kept in ice cooled water to avoid evaporation of benzaldehyde. Distilled methanol (10 ml) was added to the distillate and volume was made to 250 ml with water. The absorbance was measured at 249 nm as prescribed in AOAC procedure[2] and concentration of benzaldehyde was calculated using $E^{1\%}_{249} = 1160$. The percentage recovery in this procedure was 97$\pm$ 3.0%. (SEM; n=6)

The concentration of benzaldehyde was also determined colorimetrically by the procedure of Sanders and Schubert[3] as modified by Arya[4]. Five millilitres of the distillate was treated with 1 ml of 0.1 N HCl and 1 ml of saturated 2,4-dinitrophenylhydrazine in methanol. The mixture was heated and maintained at 60°C for 20 min in a water bath, cooled in ice cold water and treated with 5 ml of 10 per cent alcoholic potassium hydroxide solution. After 10 min, the absorbance was measured at 515 nm. A standard curve was drawn by taking known aliquots of 0.001 per cent benzaldehyde solution instead of sample distillate and developing the colour as described above. From the standard curve, the concentration of benzaldehyde was computed.

Benzaldehyde in bitter almonds is produced by the hydrolysis of amygdalin by the naturally occurring glucosidase emulsin. This hydrolysis is complete in about 1 hr at 37°C and 1.5 hr at room temperature (32°C) when powdered sample of bitter almonds is incubated with water (Table 1). Accordingly, these

TABLE 1. EFFECT OF INCUBATION ON THE LEVEL OF BENZALDEHYDE IN ALMONDS

Period of incubation (hr)	Benzaldehyde level (m/100g) *almond* 37°C	32°C
0.5	11.50	10.30
1.0	13.70	12.61
1.5	13.71	13.71
2.0	13.71	13.70

TABLE 2. CONCENTRATION OF BENZALDEHYDE IN SWEET ALMONDS ADMIXED WITH DIFFERENT LEVELS OF BITTER ALMONDS

Bitter kernel (%)	Benzaldehyde (mg/100g)* Spectrophotometric method	Colorimetric method
0	0.10$\pm$0.04	0.12$\pm$0.05
1	13.62$\pm$1.02	13.50$\pm$1.05
2	28.01$\pm$1.08	28.50$\pm$1.06
3	41.90$\pm$1.03	41.42$\pm$1.08
4	55.32$\pm$1.06	54.88$\pm$1.10
5	68.30$\pm$1.13	68.06$\pm$1.12

*Mean$\pm$SEM of 4 determinations

conditions were followed in the estimation of benzaldehyde in this investigation. In order to study the relationship between bitter kernels and benzaldehyde level in almonds, known amounts of bitter kernels were admixed with almonds. The concentration of benzaldehyde in admixtures having varying levels of bitter kernel are given in Table 2. It is apparent that concentration of benzaldehyde is directly proportional to the level of bitter kernels in sweet almonds. Concentration of benzaldehyde in all sweet almonds is very low (<0.15 mg/100 g) but when admixed with even 1 per cent bitter kernels, the level increased upto 13.5 to 14.0 mg/100 g, an almost 100 fold increase in its concentration. This makes the benzaldehyde level a very useful and reliable indicator of bitter kernels. The level of bitter kernels in commercially available sweet almonds was found to be very low (<1%) and the corresponding benzaldehyde concentration was also very low (<10 mg). In the international trade 3 per cent of the bitter kernels is permitted. Taking this into consideration, it is suggested that a maximum of 40 mg of benzaldehyde in 100 g of sweet kernels may be allowed in sweet almonds. Among the two methods, UV-spectrophotometric method is more sensitive and relatively free from interferences from other carbonyls which may be generated as a result of storage due to the autoxidation of lipids. There was practically no change in benzaldehyde level in sweet almonds upto one year's storage as indicated by the UV-spectrophotometric procedure indicating non-interference of other carbonyls in this method. On the other hand, the values obtained by colorimetric procedure were 0.12 and 0.81 mg benzaldehyde per 100 g kernels of sweet almonds when the test was performed on fresh and one year stored sample. This increase, however, is not likely to make much difference in the calculation of percentage of the bitter kernels.

We wish to thank Dr. T. R. Sharma, Director, Defence Food Research Laboratory, Mysore, for his valuable suggestion during the course of study.

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A NEW COLORIMETRIC METHOD FOR THE DETERMINATION OF CARBOFURAN, BENDIOCARB AND CARBOSULFAN

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Carbofuran (Furadan, 7-benzofuranol 2,3-dihydro 2,2-dimethyl methyl carbamate [1563-66-2]), bendiocarb (Ficam, 1,3-benzodioxol-4-ol, 2,2-dimethyl methyl carbamate [22781-23-3]) and carbosulfan (carbamic acid [(dibutylamino) thio] methyl 2,3-dihydro 2,2-dimethyl 7-benzofuranyl ester [55285-14-8]) in formulations have been determined by a new colorimetric method. Diazotized p-aminobenzoic acid was used to obtain coloured compounds with the phenols generated by hydrolysis of the carbamates with sodium hydroxide. The results show that the method could be successfully used for the determination of these active ingredients in formulations as also in contaminated water.

Carbofuran, bendiocarb and carbosulfan are broad spectrum insecticides. Carbofuran and carbosulfan are also nematocides with both contact and systemic action. These insecticides are now being extensively used for a wide variety of crops. Carbofuran, though a non-persistent type of insecticide, is highly toxic to mammals[1] and bendiocarb is poisonous to both birds and mammals[2]. The residues of these toxic carbamates enter the inland water systems: lakes, streams and ponds from the fields where they have been applied and contaminate the water environment. Literature shows that chromatographic[3-5] methods have been developed for carbofuran determination. These methods require sophisticated equipment not available in many laboratories. This necessitated a search for rapid and simple methods for their determination. Hence a colorimetric method[6] for the determination of carbofuran in formulations has been developed; diazotized sulphanilic acid was used as a coupling agent to obtain a coloured compound with the phenol, generated by the hydrolysis of the pesticide with sodium hydroxide. In this method one has to wait for half an hour to obtain the coloured compound. The spectrophotometric method of Handa[7] also needs more than 15 min to get coloured compound and the colour is stable for only 4 hr. Further bendiocarb and carbosulfan have not been determined by a colorimetric method till now. In view of the above we report here a new colorimetric procedure for the determination of the three carbamates. Diazotized p-amino-

benzoic acid has been chosen as the coupling agent.

The formulations used in the investigation included: 3 per cent granules, 50 per cent soluble powder and 75 per cent wettable powder of carbofuran, 96 per cent wettable powder of bendiocarb and 25 per cent emulsion of carbosulfan. All the materials were supplied by M/s. Rallis India Ltd. The solutions of formulations were prepared in methanol. Fifty milligram of 75 per cent carbofuran, 96 per cent bendiocarb and 25 per cent of carbosulfan were weighed into 50 ml volumetric flasks separately and dissolved in 50 ml of methanol. The stock solutions were progressively diluted with the solvent to obtain solutions of desired concentrations. The 50 per cent and 3 per cent carbofuran which contain red and violet coloured dyes, were cleaned by TLC method before the stock solutions were prepared.

Analytical grade carbofuran and bendiocarb were employed along with a relative standard of 92 per cent carbofuran to obtain standard curves. Aliquots containing 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6, and 4.0 ppm of standard carbofuran solutions were taken in clean, dry 50 ml volumetric flasks. Two millilitres of sodium hydroxide, 1 ml of 0.3% aqueous sodium nitrite and 0.5 ml of p-aminobenzoic acid were added to each flask and the solutions were made up to the mark with distilled water. Orange coloured solutions were obtained which had an absorption maximum at 490 nm. To ensure the completion of hydrolysis of the carbamate optical density values were recorded periodically at intervals of 10 min. using a Elico spectrocolorimeter (Model CL-23). The values showed that the hydrolysis was complete instantaneously. The colour was stable for 48 hr. The plot between concentration *vs* absorbance was linear over the range of 0.4 to 12 ppm for carbofuran. Similarly standard curves for bendiocarb and carbosulfan were obtained. Bendiocarb also gave an orange coloured compound with an absorption maximum at 490 nm and it was stable for 48 hr. Carbosulfan, on the other hand, gave an orange-yellow coloured compound with maximum absorbance at 475 nm and was stable for 8 hr. All the solutions of carbosulfan were made up in methanol as turbidity developed in aqueous medium. In the case of bendiocarb and carbosulfan linear relationships between concentration and absorbance were observed over the range of 1 to 16 ppm and 2 to 20 ppm respectively.

Formulations of carbofuran, bendiocarb and carbosulfan were analysed using the aforesaid procedure and the standard curves were used to determine the percentage of active ingredient.

One litre of distilled water samples were taken and spiked at concentrations of 0.3, 0.6, 0.9, 1.2 and 1.5 ppm of carbofuran, 0.6, 1.2, 1.8, 2.4 and 3.0 ppm of bendiocarb and 1.5, 3.0, 4.5, 6.0 and 7.5 ppm of carbosulfan. The pH of these samples was adjusted to 3-4 with 50 per cent sulphuric acid; 10 g of anhydrous sodium sulphate was added to each sample. The carbamates in the samples were extracted with 100 ml of chloroform by shaking for 2-4 min. Each sample was again reextracted with 50 ml of chloroform. The combined extracts were dried over 10 g of anhydrous sodium sulphate. Finally chloroform was evaporated to dryness on a water bath. Residue was dissolved in methanol. To this 2 ml of sodium hydroxide, 1 ml of sodium nitrite and 0.5 ml of p-aminobenzoic acid were

TABLE 1. ANALYSES OF COMMERCIAL FORMULATIONS OF CARBOFURAN, BENDICOARB AND CARBOSULFAN

Sample	Carbofuran			Bendiocarb	Carbosulfan
	Granules 3%	Soluble powder 50%	Wettable powder 75%	Wettable powder 96%	Emulsion 25%
1	2.86	49.34	73.66	93.33	24.00
2	2.98	49.57	74.13	94.55	24.46
3	3.00	49.68	74.49	95.09	25.00
4	2.93	49.74	74.57	95.34	24.49
5	2.92	49.79	75.00	95.48	24.59
6	2.95	50.00	74.35	95.58	24.83
7	2.96	49.68	74.68	95.63	24.86
Mean	2.94	49.69	74.41	95.00	24.60
SD	± 0.05	± 0.20	± 0.43	± 0.82	± 0.33

TABLE 2. RECOVERY OF CARBOFURAN, BENDIOCARB AND CARBOSULFAN FROM SPIKED WATER SAMPLES

Sample	Carbofuran			Bendiocarb			Carbosulfan		
	Added (ppm)	Found (ppm)	Recoveries (%)	Added (ppm)	Found (ppm)	Recoveries (%)	Added (ppm)	Found (ppm)	Recoveries (%)
1	0.3	0.29	96.7	0.6	0.58	97.0	1.5	1.46	97.3
2	0.6	0.57	95.0	1.2	1.12	98.3	3.0	2.94	98.0
3	0.9	0.88	97.8	1.8	1.75	97.2	4.5	4.39	97.5
4	1.2	1.17	97.5	2.4	2.35	97.9	6.0	5.70	95.0
5	1.5	1.47	98.0	3.0	2.90	96.7	7.5	7.34	97.9

added, colour was developed and absorbance recorded.

Analytical data pertaining to the analyses of the formulations of carbofuran, bendiocarb and carbosulfan are given in Table 1. The results indicate that the percentages of the active ingredient in the formulations of the carbamates are in agreement with those given on the labels. Further, the colorimetric procedure reported here is more suitable than those reported by Mithyantha[6] and Handa[7]. In the present method the colour develops instantaneously and remains stable for longer period. The results in Table 2 indicate that the recoveries of the three carbamates from the spiked water samples ranged from 95 to 98 per cent and are comparable to those reported by Handa[7]. Hence it may be concluded that the present method is suitable both for the determination of active ingredients in formulations as also in water contaminated with them.

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INCIDENCE OF CLOSTRIDIA WITH REFERENCE TO DAIRY PROCESSING

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A total of 98 samples of cow and buffalo raw milk, pasteurized cow milk and processed cheese were analysed for total clostridial and spore counts using MPN method, which ranged between 0 and 460/100 ml of milk or/g of cheese. The average counts of clostridial spores in raw cow, and buffalo milk and pasteurized cow milks were 9, 26 and 19/100ml, respectively, whereas in processed cheese the average spore count was 2/g.

Clostridia are present in canned dairy products such as processed cheese and cheese spread by virtue of their anaerobic, spore forming and heat resistant characteristics[1,2]. They spoil the dairy products either due to their predominantly saccharolytic or proteolytic activities or both[3-5]. In addition, the pathogenic strains of clostridia as live cells or their toxins have been the subject of several investigations[6-11]. *Clostridium sporogenes*, *C. butyricum*, *C. perfringens* and *C. botulinum* pose a potential risk as spoilage or pathogenic organisms in milk and milk products. These organisms gain access as vegetative cells or spores into milk and milk products through various sources such as soil, air, water and feeds[12]. In the present investigation, an attempt has been made to estimate the total and spore

counts of clostridia at some selected stages of dairy processing such as raw milk, pasteurized milk and processed cheese.

Samples: Raw whole milk of cows and buffaloes, HTST (72°C/15 sec) pasteurized cow milk and processed cheese collected from the Institute Dairy were examined for total clostridial and spore counts.

Enumeration of clostridia and their spores: For enumeration, all milk samples were examined as such or dilutions were made with 0.1 per cent peptone, if required. Processed cheese homogenates were prepared from 11 g of cheese and 99 ml of 2 per cent sodium citrate solution at 45°C. Subsequent dilutions were in 0.1 per cent peptone. Clostridia were enumerated using most probable number (MPN) in differential reinforced clostridial medium (DRCM)[13]. The medium contained: peptone, 10g; lab lemco, 10g; hydrated sodium acetate, 5g; yeast extract, 1.5 g; soluble starch, 1g; glucose, 1g; L-cysteine-HCl, 0.5g; distilled water, 1000ml. Additions were made aseptically to give anhydrous sodium sulphite, 0.04% and ferric citrate, 0.07 per cent. Total counts of clostridia were determined directly without heating[14]. Determination of spore counts of clostridia was done after heating the milk samples or cheese homogenates at 80°C for 10 min[15]. Inoculated DRCM tubes were incubated at 37°C up to 7 days. Confirmation of presumptive tubes was done by heating at 80°C for 10 min and inoculating into tubes containing fresh medium. Cultured tubes indicating positive results were blackened. Microscopic examination of stained spores was also carried out.

Results on total clostridial and spore counts of raw cow and buffalo milks, pasteurized cow milk and processed cheese samples have been presented in Table 1. Raw cow and buffalo milks had a total clostridial counts of 0-460/100 ml as compared to spore counts of 0-150/100 ml, indicating contamination by vegetative cells of clostridia in addition to their spores. Further, concentration of spores was more in raw buffalo milk than in cow milk. This may be due to the poor bacteriological quality of buffalo milk compared to cow's milk. These data indicate that stringent measures are required during production of milk since contamination by spores of clostridia at the time of milk production continue to resist pasteurization temperature as evident by 9/100 ml and 26/100 ml of spores in raw cow and raw buffalo milks, respectively. Almost same number of spores also prevailed (19/100 ml) even after pasteurization. Donnelly and Busta[5] have indicated that milk is perhaps the most protective medium for heating spores. The number of spores (19/100 ml) in pasteurized milk is more or less similar to the number of spores (2/g) in processed cheese in view of ten times concentration of the yield during the manufacture of cheese[2], indicating that spores of clostridia in contaminated raw milk including cheese milk escape the destruction during processing steps and are finally carried to the canned products. The large number of spores detected in processed cheese (460/g) may be considered as potential cause for spoilage by late blowing[14].

TABLE 1. TOTAL AND SPORE COUNTS OF CLOSTRIDIA IN MILK AND PROCESSED CHEESE

Sample	No. of samples	Total clostridial count	Spore count
Raw cow milk (per/100 ml)	38	51 (0–160)	9 (0–91)
Raw buffalo milk (per/100 ml)	20	74 (0–430)	26 (0–150)
Pasteurized cow milk (per/100 ml)	20	22 (0–360)	19 (0–150)
Processed cheese (per/g)	20	2 (0–460)	2 (0–460)

*Average based on geometric mean count. Figures in parantheses indicate the range.

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MATURITY STANDARDS FOR FRUITS OF PICKLE MANGOES

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In mango var 'Dedseri' changes in fruit volume, total soluble soilds content and acidity were recorded at four days interval starting from April 9 to June 12. Pickling of mangoes was done from those, harvested on May 15, May 29 and June 12. The pickles organoleptically evaluated after 3, 6 months storage showed no differences. After 12 months storage those pickled from less mature fruits were found to be better. In the two earlier picklings pulp texture and adherence of palp to the skin and endocarp were better. The best stage of harvest for pickling appears to be just after the endocarp starts hardening.

Determination of correct period of harvest of mango for pickling is of prime importance as the quality of the pickle is greatly influenced by the proper stage of maturity of the fruits. Maturity standards for table and juicy varieties were worked out by many workers but for pickle mangoes not much information is available.

Period of optimum maturity varies with varieties and as such there is need to have maturity standards for different pickle varieties. The present investigations were carried out to identify the proper stage of maturity of fruits of pickle cultivar 'Dedseri' under Sangareddy conditions and also to study the span of harvesting period without effecting quality.

Thirty uniform fruits at marble stage were selected in each of the two trees and their growth and development were studied. Fruit colour, bloom, prominence of beak, endocarp hardening, total soluble solids (TSS) and acidity were recorded by harvesting two fruits from each of the two trees at regular intervals. Pickling was done thrice at fortnightly intervals on May 15th, 29th and June 12th. Pickle of *Avakaya* type was prepared. The evaluation of pickles was done after 3, 6 and 12 months of storage for their keeping quality by a panel of six trained judges.

It is evident from the data that the rate of acid loss of the fruits started decreasing from 2nd fortnight of April and start of endocarp hardening was followed by cessation of increase in fruit volume and build up of total soluble solids content. Beak became less prominent and green colour of the fruit turned lighter. First pickling was done at this stage and then subsequently twice at fortnightly intervals.

The results of evaluation of pickles after 3rd and 6th month of preservation, revealed that no differences were perceptible among pickles prepared on different dates. After 12 months of storage, the earlier picklings were found better than the last one in which the fruit pieces were slightly darkened. Pulp texture and adherence of pulp to the skin were better in the two earlier picklings. Sastry and Krishnamurthy[2] and Sastry *et al*[3] had observed that the fruits at early maturity (8-9 weeks growth) stage were best suited for pickling. When mangoes reached almost maximum size and weight, the pickles had good colour, texture and flavour with predominant mango flavour.

For pickling best stage of harvest appears to be after endocarp hardening when there is not much reduction in acid content and not much build up of soluble solids. Presence of maximum starch content at this stage may be helpful in texture retention. However harvesting for pickling can be delayed close (say 10 days earlier) to physiological maturity of fruits. Under Mysore conditions Pruthi and Bedeker[1] also did pickling successfully over a period of 2 months. (April 23rd to June 18th). In the present studies pickles made from fruits harvested at advance maturity had a shorter shelf life.

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TABLE 1. PHYSICAL CHARACTERS AND CHEMICAL ANALYSIS OF 'DEDSERI' VARIETY MANGO

Harvest time		Fruit vol. (ml)	TSS (°Brix)	Acidity (% citric)	Remarks
Month	date				
April	7	106	8.75	3.76	
	11	137	8.58	3.26	
	15	162	8.38	3.20	Beak prominent
	19	176	8.25	3.13	Beak less prominent Fruit green
	23	194	8.25	3.10	Stone hardening starts
	29	207	8.13	3.10	
May	1	190	8.45	3.04	
	5	197	8.95	3.00	Stone hardened
	9	197	8.90	2.88	
	15	198	9.13	2.95	First pickling
	22	198	9.88	2.88	
	29	198	10.13	2.63	Second Pickling
June	5	198	10.88	2.65	
	12	198	11.08	2.53	Third Pickling

Mean values for two trees;

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NUTRITIONAL VALUE OF KER (*CAPPARIS DECIDUA*) FRUIT

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Unripe fruit of Ker (*Capparis decidua* Edgew) consumed by local population in many forms, was studied for its nutritional value. On dry matter basis, it contained 14.88% protein, 7.43% fat, 5.96% total mineral matter, 12.32% crude fibre and 59.41% digestible carbohydrates. The contents of β-carotene, ascorbic acid, calcium, phosphorus, iron, copper, zinc and manganese were 5.40, 120.70, 90, 179, 3.5, 1.1, 1.6 and 1.9 mg/100g respectively. Albumin, globulin, prolamine and glutelin fractions formed 53, 16, 11 and 12 % respectively of total protein in the fruit. The fruit was free from tannins, trypsin inhibitor and haemagglutinin activity and had 304 mg/100g of phytic acid content.

Ker (*Capparis decidua* Edgew) is a perennial woody plant which grows wild on barren soils of arid and semi-arid regions of India. On account of its deep tap root system, scanty foliage, mucilaginous sap and tough conical spines the plant has acquired special xerophytic adaptive mechanism and thrives under extreme dry and hot climate. Berry-shaped unripe fruits, commonly known as Teent, are 0.7-1.5 cm in diameter and contain many seeds. The skin of the fruit is thick, bony, shiny and dark green. The fruits contain golden yellow pulp and white seeds. All the parts of the fruit are edible. The unripe fruits, generally consumed as vegetable and pickles, form an integral part of the diet of the people in desert and semi-desert areas of the country. Information on chemical composition and nutritional value of the fruit, which may contribute significantly to the nutrient intake of local population, is lacking. The present study was on the chemical make-up and nutrient profile of the unripe fruit.

Unripe fruits of medium size were selected and after removing the stalks fresh fruits were used for the estimation of ascorbic acid and moisture. For other determinations the fruits were dried at 45°C, ground and then used. Analysis for moisture, ash, crude fibre, crude protein, calcium, phosphorus and ascorbic acid was done by standard methods[1]. For estimating the crude protein content the N value was multiplied by 6.25. Crude fat was determined by Soxhlet extraction using petroleum ether (b. p. 40-60°C) as solvent[2]. Total digestible carbohydrates were calculated by subtracting the percentage of crude protein, fat, crude fibre and ash from 100. β-Carotene was extracted from ground fruits by refluxing in a mixture of acetone

and hexane (3:7 V/V), separated on alumina column and determined spectrophotometrically.[3] Iron, zinc, copper and manganese were determined by atomic absorption spectrophotometry[1].

Different solubility fractions of protein were isolated in water, one per cent sodium chloride solution, 60 per cent ethanol and 0.4 per cent sodium hydroxide[4] respectively and their nitrogen content was determined by Kjeldahl method.

Phytic acid was extracted with 0.5 M nitric acid and estimated by the method of Davies and Reid[5]. Procedure employed by Roy and Rao[6] was followed for assaying trypsin inhibitor activity using casein as substrate. For detecting the haemagglutinin activity the method of Liener[7] was adopted. Tannins were estimated by the modified vanillin-HCl method of Price *et al.*[8]

Table 1 shows the proximate composition, ascorbic acid and β-carotene contents of the unripe fruit. Levels of protein, fat, crude fibre and total minerals were high when compared with values reported for some common fruits and vegetables[9]. The fruit seems to be a good source of ascorbic acid and β-carotene. Fifty grams fruit (moisture free) will provide 60.35 mg of ascorbic acid and 2.7 mg of β-carotene which is more than the daily Recommended Dietary Allowance (RDA) for ascorbic acid (50 mg) and 90 per cent of RDA for vitamin A (3 mg β-carotene)[9]. Since dietary fibre, when used at adequate levels, has been shown to have a number of beneficial effects[10], the *C. decidua* fruit containing considerable amounts of crude fibre will have additional utility when included in low fibre diets.

Table 2 summarises the amounts of the minerals present in the fruit. An intake of 100 g of fruit in the adult diet will supply about 20 per cent of the RDA for calcium[9] and 3.5 mg iron. The fruit contained higher concentrations of phosphorus and copper than those found in common Indian fruits like apple, banana, guava and mango[9]. Levels of zinc and manganese in the fruit were also high.

Data on the solubility fractions of protein of the fruit are presented in Table 3. The presence of albumin as a predominant protein fraction (52.76%) is suggestive of good quality protein[11]. Utilization of vegetable protein for human nutrition is hampered by the presence of trypsin inhibitor, haemagglutinins (lectins) and tannins[12]. The fruit did not contain any of these antinutritional factors.

The fruit contained 304 mg/100 g of phytic acid which is a quite high concentration as compared to 54 mg/100 g in guava[9]. This level of phytic acid may interfere with the availability of minerals.

From the limited data obtained it may be concluded that *C. decidua* fruit, a rich source of ascorbic acid, β-carotene, minerals and richer in protein than the common fruits and free from antinutritional factors, merits attention by agricultural and food scientists. It seems to have immense potential as a source of nutrients for the inhabitants of areas prone to extreme drought and hot conditions where other major food crops cannot be grown.

TABLE 1. PROXIMATE COMPOSITION AND VITAMIN CONTENTS OF UNRIPE FRUITS OF CAPPARIS DECIDUA

Constituent	
Moisture (%)	28.00
Crude protein (%)	14.88
Ether extractives (%)	7.43
Crude fibre (%)	12.32
Ash (%)	5.96
Digestible carbohydrates (%)	59.41
Ascorbic acid (mg/100 g)	120.70
β-carotene (mg/100 g)	5.40

Figures are means of six values expressed on dry matter basis.

TABLE 2. MINERAL COMPOSITION OF UNRIPE C. DECIDUA FRUITS

Mineral	(mg/100g)
Calcium	90
Phosphorus	179
Iron	3.50
Zinc	1.60
Copper	1.10
Manganese	1.90

Figures are means of six values expressed on dry weight basis.

TABLE 3. CONTENTS OF VARIOUS PROTEIN FRACTIONS OF UNRIPE FRUITS

Protein fraction	(g/100g)*
Albumin	7.85 (52.76)
Globulin	2.40 (16.13)
Prolamine	1.58 (10.62)
Glutelin	1.79 (12.03)
Residue	1.26 (8.46)

Figures are means of four values. Figures in parentheses are percentages of total protein.
*Dry matter basis.

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PREPARATION AND CHARACTERIZATION OF FERRIC LACTOSE

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Ferric lactose complex which appears to be a single molecular entity containing 25% iron and 30% lactose, was found to consist of several iron complexes with isomeric forms of diketoses and their corresponding acids, further stabilized by 30% extra lactose, probably in the form of a complex. The nature of these complexes was studied using techniques such as dialysis, thin layer chromatography, column chromatography and U. V. absorption.

Ferric lactose prepared by a method similar to that for ferric fructose[1] has been found to be suitable for fortification of milk with iron.[2] As this complex has not been described earlier, an attempt has been made to characterize it.

Ferric lactose was prepared by mixing equal volumes of 0.1 M ferric chloride and 1 M lactose at room temperature. pH was adjusted to 11 with 1 N NaOH. At neutral pH, precipitates of $Fe(OH)_3$ were observed which were solubilized by the addition of more NaOH. To the clear solution, absolute ethanol (four times the volume of $FeCl_3$ or lactose) was added to precipitate the complex from its sequestered solution. Precipitates of ferric lactose were centrifuged and dissolved in the minimum amount of water; pH was again adjusted to 11 and the complex was reprecipitated with ethanol. Precipitates were separated by centrifugation and washed with alcohol followed by acetone. Ferric lactose complex thus formed was dried over anhydrous $CaCl_2$ at room temperature in a vacuum desiccator. The complex was stored at 4°C in brown bottles; it was analyzed for iron[3] and sugar[4] according to the reported methods. The nature of the complex was studied by dialysis, thin layer chromatography (silica gel, 250μ), column chromatography on Sephadex-G 25 and absorption spectroscopy (Bausch & Lomb Spectronic-2000).

The complex was found to contain 25 per cent iron and 30 per cent lactose.

Concentrated solutions (5-10 mg/ml) of the ferric lactose complex were fairly stable, whereas dilute solutions in water (0.01 per cent) kept at room temperature or even in a refrigerator at 4°C gave a sediment. It is clear that sedimentation of the complex is directly related to the dilution of the solution and inversely related to temperature (Table 1). The finding of 30 per cent lactose in the supernatant (Table 2) indicated that lactose was only loosely bound to the complex and was essential for its solubility and stability.

Presence of loosely bound lactose which separated in solution was also confirmed by TLC and sephadex chromatography. On chromatoplates sprayed with benzidine a yellow spot having the same R_f value as standard lactose was observed. No free ferric ions were traceable by potassium thiocyanate hydrochloric acid spray.

TABLE 1. BEHAVIOUR OF AQUEOUS SOLUTIONS OF FERRIC LACTOSE ON KEEPING AT ROOM TEMPERATURE OR STORING AT 4°C

Ferric lactose concn (mg/ml)	Temp. (°C)	Settling time (days)
1.0	31	1
1.0	4	40
4.0	4	150

TABLE 2. ANALYSIS OF SUPERNATANT OF AQUEOUS SOLUTION OF FERRIC LACTOSE* AT 30°C ON DRY WEIGHT BASIS

Time interval (hr)	Fe^{3+} (%)	Lactose (%)	Settles or not
0	25	30	No
2	25	30	No
4	25	30	No
6	25	30	No
12	25	30	No
18	15	30	Yes
24	0	30	Yes, Completely

*1 mg/ml.

Examination of the sedimented complex (after removal of lactose) indicated that a part of the complex contained a Saliwanoff positive compound, which was presumably lactulose obtained from isomerization of aldose (glucose unit of galactose) to ketose in the presence of alkali. The isomerization of glucose unit of lactose to mannose type configuration cannot also be ruled out.

The sedimented complex was washed with distilled water and dissolved in 1 N hydrochloric acid (separate studies on complex have shown that complex starts dissociating at pH 1.5 but is stable at pH above this).

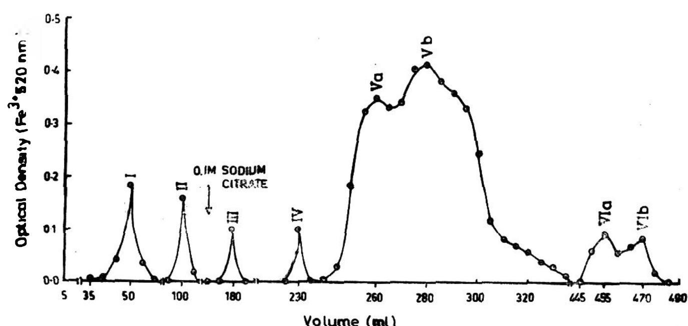

Fig 1. Sephadex G–25 column chromatcgram of ferric lactose complex (column size 1.4 x 62.5 cm, rate of flow 30ml/hr. R.T. 22°C.)

λ max of fractions: I. 260–280; III. 200–230; v_a, v_b 340 nm.

A chromatogram of the residue of the complex in 1 N hydrochloric acid showed the presence of free acids after developing in n-butanol: acetic acid: water (4:1:1) and spraying with litmus. These acids were identified as lactonic and gluconic acids. Lactonic acid could arise possibly from alkaline iron oxidation of lactose and the former compound was then hydrolysed in presence of acid to give gluconic acid.

These studies indicate that ferric lactose complex is not a single entity, but is composed of different molecular complexes of iron with different isomeric and oxidized forms of lactose (lactulose and lactonic acid), which are stabilized by the presence of lactose.

In order to fractionate different molecular species of the complex, it was eluted from sephadex G-25 column with water, but only lactose was eluted (in the same elution volume as standard lactose) and the remaining complex could not be eluted from the column with water or even by changing the pH of water gradually to 10.

However, complex iron could be fractionated into six fractions on Sephadex G-25 column by serial elution with 0.1 N NaOH, 0.1 M sodium citrate, water and 0.1 N HCl (Fig 1). The complex lactose appeared as a single peak corresponding to that of standard lactose.

A strong absorption at 260-280 nm by fraction I indicates the presence of a carbonyl group in the compound (Fig 1). This might also be responsible for the Saliwanoff positive reaction in the sediment of ferric lactose complex. Fraction V exhibited an absorption peak at 340 nm, which was similar to the oxygen bridge maxima, reported in iron complexes[3] (cf. ferric fructose). Fraction III showed a broad absorption peak between 200 and 230 nm region. The nature of this absorption peak is not known.

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STUDIES ON ACCEPTABILITY AND AVAILABILITY OF FORTIFICANTS PRESENT IN MILK AND WHEY

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Milk and whey fortified with ferric lactose and ascorbic acid were found to be highly acceptable. Chemical analyses indicated that the fortificants were present in biologically available forms. The keeping quality of fortified samples was comparable to the corresponding controls.

Iron deficiency is one of the most common diseases prevalent in the world. A persistent problem in implementing iron fortification programmes is the selection of low cost iron salt with consumer acceptability and biological availability of iron. It was, therefore, planned to fortify milk and whey with iron and vitamin C (ferric lactose and vitamin C). The present study deals with consumer acceptability of these fortified foods and their analyses for iron and vitamin C.

Fresh buffalo milk collected from a single source was boiled and kept in a refrigerator for 1½ hr. Cream layer was removed and skim milk was used for fortification. Whey was prepared from skim milk by the addition of tartaric acid (900 mg/kg. milk).

Ferric lactose was prepared according to the method of Saltman *et al.*[1] with a slight modification. All other reagents used in the study were of analytical grade. For fortification, 25 mg of ferric lactose (6.25 mg of iron) and 25 mg of ascorbic acid dissolved in 5 ml of water were added to 95 ml milk or whey. Organoleptic evaluation of the fortified foodstuffs was done by a panel of judges using a 7-point scale score card which was prepared from Hopkins 11-point score card[2].

TBA Test with milk and whey was performed by the method of King.[3] In milk and whey (after protein precipitation) ferrous iron was analysed by α, α'' dipyridyl method[4]. For estimation of total iron, sodium sulphite was added to the reaction mixture before adding dipyridyl reagent. Vitamin C was estimated by DNP method[5]. Ascorbic acid, dehydroascorbic acid and diketogulonic acid were separated by paper chromatography using n-butanol: acetic acid: water (4:1:5); 2, 6-dichlorophenolindophenol and phenyl hydrazine were used as spraying agents.

Fortification with ferric lactose and ascorbic acid did not show any marked effect on appearance, colour consistency, taste and flavour of milk and whey (Table 1). Hence, the overall score was also not affected and the products were rated to be almost 'very good' and of course acceptable.

There was no change in the keeping quality of fortified milk and whey (when kept at 4°C for 3 days) as compared to their corresponding unfortified samples and freshly prepared fortified samples. TBA value of milk decreased after fortification (Table 1). This is in contrast to earlier studies where it had been found that ferrous iron was more detrimental to lipid oxidation as compared to ferric iron[6,7]. The absence of oxidative deterioration is not due to absence of fat as has been shown earlier during studies on fortification of whole milk with ferric lactose and ascorbic acid[8]. This may be explained by the antioxidant action of ascorbic acid.

In fortified milk and whey, 93.6 and 94.2 per cent of the added iron was present in the ferrous form

TABLE 1. ORGANOLEPTIC EVALUATION AND TBA VALUES OF MILK AND WHEY FORTIFIED WITH FERRIC LACTOSE AND VITAMIN C

Treatment	Appearance	Colour	Consistency	Taste	Flavour	Overall score	TBA value *Mean±S.D.
				Milk			
Unfortified	5.84	5.87	6.00	5.75	5.75	5.84	0.039±0.0020
Fortified	5.50	5.50	5.90	5.75	5.62	5.65	0.01 ±0.0010
				Whey			
Unfortified	5.77	5.87	5.88	5.66	5.70	5.77	0.04 ±0.0015
Fortified	5.77	5.77	5.88	5.24	5.66	5.75	0.038±0.0018

Key: Perfect-7, Very good-6, Good-5, Average-4, Fair-3, Bad-2, Inedible-1.
*Mean of 3 observations.

TABLE 2. AVAILABILITY OF IRON AND VITAMIN C FROM FORTIFIED MILK AND WHEY

Sample	Iron* (mg/100 ml)	% available iron	Vitamin C (mg/100 ml)			% retention of total vitamin C
			Total	Dehydroascorbic acid	Ascorbic acid	
	Mean±S.D.		Mean±S.D.	Mean±S.D.	Mean±S.D.	
			Milk			
Unfortified	0.00	—	1.0±0.04	0.82±0.02	0.18	—
Fortified	5.85±0.210	93.6	26.0±0.06	26.00±0.15	0.00	100
			Whey			
Unfortified	0.00	—	0.7±0.12	0.55±0.10	0.15	—
Fortified	5.90±0.095	94.2	25.7±0.13	25.70±0.04	0.00	100

Values are the mean of 6 observations
*Iron in ferrous form.

respectively (Table 2). This is the form of iron which can be absorbed at any level of the gut if placed in direct contact with the mucosa[9].

By DNP method, 100 per cent of the added ascorbic acid could be recovered from the fortified samples (Table 2). However DNP method did not distinguish between dehydroascorbic acid and diketogulonic acid. Absence of diketogulonic acid, the biologically non-available form of vitamin C[10] in the fortified samples was confirmed by paper chromatography. In the fortified samples, vitamin C was found to be present as dehydroascorbic acid. This indicated that ascorbic acid was oxidized to dehydroascorbic acid with simultaneous reduction of ferric to ferrous iron.

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STUDIES ON THE DEVELOPMENT OF CHICKEN SKIN AS A SNACK

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A crisp chicken skin snack was developed using different processing techniques. The final product had an average proximate composition of: moisture 4.3%, protein 43.49% and fat 47.69%. Organoleptic studies of the product showed good to very good scores for general appearance, flavour, crispiness and overall acceptability.

Chicken skin constitutes approximately 11 to 14 per cent of the whole carcass[1,2]. Consumers in India generally do not prefer poultry meat with skin on. In the manufacture of several processed poultry products, skin is left behind as a byproduct. These facts reflect that substantial quantities of raw chicken skin would be available from organised poultry dressing and processing plants in the country and could be used for developing acceptable products. Some workers have tried to study the characteristics of cooked chicken

skin[3,4,5]. In the present study, different processing techniques have been studied to develop acceptable crisp snack from cured chicken skin.

Skins were collected from well synged broiler carcasses and cleaned to remove fatty deposits from the subdermal layer. A known quantity of skins were cured in 2 per cent sodium chloride solution at 4°C for 14 hr and pick up in weight was recorded. Cured skins were subjected to the following treatments:

A. Cured+fried
B. Cured+pressure cooked+fried
C. Cured+pressure cooked+oven dried+fried
D. Cured+oven dried+fried
E. Cured+pressure cooked+minced+fried

Cured skins were cut directly into small rectangular pieces (about 1×5 cm)in treatments A and D, and after pressure cooking (4 min at 1.02 kg/cm^2) in treatments B and C. Oven drying of the skins was done at 120°C for 2 hr. Pressure cooked, cooled skins in treatment E were minced through a meat grinder having 4 mm plate. The skins from all the treatments were fried separately in hydrogenated vegetable oil at 160°C for 4-6 min. After draining and cooling, the skins were finally fried at 200°C for 1-2 min to produce puffed appearance and crisp texture. The final product was subjected to organoleptic evaluation for general appearance, flavour, crispiness and overall acceptability on a 7-point Hedonic scale. The proximate composition of chicken skin snacks obtained from different treatments was estimated as per AOAC[6] methods.

The material losses during cleaning of raw skins amounted to 17.1 per cent while weight gain during overnight curing was 17.65 per cent. Pressure cooking of the cured skins in treatments B, C and E resulted in an average cooking loss of 40.95 per cent. Zabik[5] reported a pressure cooking loss of 46.59 per cent in chicken thigh skin pieces. Average yields of the final products from various treatments ranged between 18.25 and 24.33 per cent (Table 1). In an earlier study on chicken skin as a snack Goode and Cooper[4] reported 25.7 per cent average yield of cooked chicken skins.

The composition of the raw chicken skin is: moisture 52.25, protein 8.01 and fat 38.20 per cent whereas that of chicken skin snack from different treatments averaged: moisture 4.31, protein 43.49 and fat 47.69 per cent. It can be observed from Table 1 that the moisture content of the chicken skin has decreased drastically and the protein content has increased substantially. Though all the treatments showed an increase in the fat content of the final product, the fat pickup was maximum in treatment E where mincing of the pressure cooked chicken skin increased the surface area and porosity.

TABLE 1. PROXIMATE COMPOSITION AND COOKED YIELDS OF CHICKEN SKIN SNACK

Treatments	Moisture (%)	Crude fat (%)	Protein (%)	Cooked yields (%)
A	4.54±0.12	46.26±0.13	44.32±0.10	24.33±0.83
B	4.40±0.05	47.29±0.08	44.03±0.02	19.17±0.41
C	4.15±0.08	47.04±0.02	43.80±0.02	22.50±0.15
D	4.16±0.19	47.63±0.06	43.94±0.02	18.25±0.37
E	4.33±0.22	50.23±0.47	40.87±0.16	22.19±0.42

Values are mean±SEM of 4 samples

TABLE 2. MEAN ORGANOLEPTIC SCORES OF CHICKEN SKIN SNACK

Treatments	Appearance	Flavour	Crispness	Overall acceptability
A	6.25±0.46	5.80±1.09	5.38±1.60	5.85±0.99
B	4.50±1.20	4.80±1.10	5.88±1.25	5.25±1.39
C	6.25±1.39	5.80±1.64	5.63±0.92	6.13±0.99
D	3.88±1.64	4.60±1.52	5.13±2.10	4.63±2.26
E	4.89±1.05	5.33±0.87	5.22±0.67	5.00±1.22

Results are expressed on a 7-point hedonic scale 7=Excellent, 1=Extremely poor.

The results of organoleptic studies on chicken skin snacks (Table 2) indicate consistently good to very good scores with respect to general appearance, flavour, crispness and overall acceptability of the products from treatments A, B, C and E. Chicken skin snack from treatment C involving curing, pressure cooking, oven drying and frying was most acceptable whereas that from treatment D where pressure cooking was not done was least acceptable especially in its general appearance.

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EVALUATION OF VARIETY MEATS FROM BUFFALOES FOR SOME QUALITY PARAMETERS

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Low cost variety meats comprising head meat, tripe and heart from buffaloes were evaluated for compositional and functional properties and were compared with the thigh (carcass) meat. Protein % was higher in the thigh and the head meats while fat % was higher in the heart. Non protein nitrogen % was higher in the thigh meat and hydroxyproline % was higher in the head meat indicating high collagen content. pH was significantly different in all the 4 meats and head meat and tripe had higher pH resulting in higher water holding capacity (WHC). Heart showed the lowest WHC with negative value. Cooking release volume did not differ significantly while emulsifying capacity was significantly higher in head and thigh meats. In quality head meat ranked better than tripe and hear: and is comparable to thigh meat.

Variety meats are the low cost edible byproducts which are generally used in sausages and cooked specialities. Considerable quantities of the variety meats produced in our country are being used in the traditional manner[1]. Their use in comminuted meat products may not only bring more returns but also improve their palatability and utilization. Knowledge on chemical composition and functional properties of these variety meats is essential for considering their inclusion in comminuted meat products. Physico-chemical constants of various meats for use in sausage formulation were reported[2]. No information is available on the quality characteristics of variety meats from buffaloes. The present study was carried out to evaluate and compare head meat (from pole or crown of head), tripe (paunch), heart and thigh meat (carcass meat) for some compositional and functional properties.

Four samples each of thigh meat, head meat, tripe and heart were collected from the local buffalo meat market and were frozen at-10°C for 20 hr before being thawed, minced and analysed. Separable fat and connective tissue were removed before mincing. Samples were analysed for proximate composition[3] and fractionation of sarcoplasmic and myofibrillar proteins[4]. Extractable proteins were calculated as total of sarcoplasmic and myofibrillar proteins and expressed as per cent of total proteins. Non-protein nitrogen was estimated by the procedure of Hegarty *et al.*[5] and hydroxyproline content was estimated by the method of International Organization for Standardization[6]. The following functional properties were determined: pH (by Toshniwal combined electrode pH meter), water holding capacity (WHC) by the centrifugation method of Hamm[7] as modified by Wardlaw *et al.*[8], emulsifying capacity (EC) as per Swift *et al.*[9] and cooking release volume (CRV) by heating 20 g sample in a polythene bag at 80°C for 20 min in a water bath and estimating the per cent loss in weight after draining out exudate. Results were analysed for significant differences by Duncan's multiple range test[10].

Results of proximate composition and fractionation of proteins are presented in Table 1. No significant differences were found in the moisture content between the meats. Due to relatively low per cent of fat com-

TABLE 1. PROXIMATE COMPOSITION AND PROTEIN FRACTIONS (PER CENT) OF THIGH AND VARIETY MEATS FROM BUFFALOES

	Thigh meat	Head meat	Tripe	Heart
Moisture	77.47±1.22	76.44±0.74	81.18±0.92	79.83±2.15
Protein	20.76±0.45^{cd}	21.80±0.77^{ab}	17.42±0.85^{ac}	17.24±0.89^{bd}
Fat	0.91±0.05^{a}	1.69±0.29^{b}	1.11±0.29^{c}	5.02±1.31^{abc}
Ash	1.38±0.14^{abc}	1.02±0.04^{a}	1.03±0.03^{b}	0.99±0.01^{c}
Sarcoplasmic proteins	4.18±0.48	3.48±0.64	4.35±0.23	2.87±0.12
Myofibrillar proteins	6.30±1.32	5.11±0.13	4.49±0.70	4.53±0.27
Extractable proteins	50.05±7.43	39.72±3.84	46.98±1.64	43.39±2.78
Non-protein nitrogen	0.28±0.04^{abc}	0.16±0.02^{ae}	0.11±0.04^{bde}	0.20±0.02^{cd}
Hydroxyproline	0.15±0.03^{cd}	0.54±0.02^{abc}	0.41±0.01^{ade}	0.17±0.02^{be}

Means (±SE) in each row with the same superscript differ significantly ($P<0.05$)

TABLE 2. FUNCTIONAL PROPERTIES OF THIGH AND VARIETY MEATS FROM BUFFALOES

Type of meat	pH	Water holding capacity (ml/100g)	Cooking release vol. (g/100g)	Emulsifying capacity (ml/2.5g)*
Thigh	5.59 ± 0.07^a	1.64 ± 6.29^b	34.86 ± 1.86	128.75 ± 9.15^{cd}
Head	6.43 ± 0.09^a	33.60 ± 9.31^{ab}	32.04 ± 1.49	134.25 ± 4.99^{ab}
Tripe	6.90 ± 0.07^a	20.31 ± 7.92^c	35.39±3.[illegible]6	103.25 ± 5.94^{bd}
Heart	5.98 ± 0.08^a	-17.43 ± 1.82^{ac}	39.83±[illegible].85	101.25 ± 3.84^{ac}

*ml of oil emulsified/2.5g meat.

Means in each column with the same superscript differ significantly ($P < 0.05$)

pared to beef slightly higher values for moisture content were observed and a relatively higher per cent in tripe might be due to the initial washing of samples to remove dirt that sticks due to handling practice. Thigh meat and head meat had significantly higher per cent protein than tripe and heart and the values were comparable to the values reported for cow meat by Wiley *et al.*[11] Per cent fat was significantly higher in the heart whereas ash content was higher in the thigh meat. The low fat per cent in buffalo meat might be due to the poor marbling reported in buffalo carcasses[12].

There were no significant differences among the meats with respect to sarcoplasmic proteins, myofibrillar proteins and extractable proteins of total proteins; however, thigh meat showed relatively higher values. Tripe and heart which are smooth and cardiac muscles respectively had relatively lower per cent of myofibrillar proteins than the thigh and head meats which are skeletal muscles. And skeletal muscle proteins were reported to have better functional properties compared to smooth and cardiac muscle proteins[13]. Non-protein nitrogen was maximum in thigh meat and differed significantly from others. Hydroxyproline per cent was significantly higher in head meat and tripe compared to others indicating higher collagen content and the values were higher than those reported by Wiley *et al.*[11] for cow meat and head meat. Connective tissue proteins were reported to possess water-binding mechanisms similar to those used by myofibrillar proteins[7].

Results of functional properties are presented in Table 2. pH differed significantly among the meats. Tripe and head meat showed relatively higher pH than heart and thigh meat. WHC was significantly higher in head meat followed by tripe while heart showed a negative value. High WHC in head meat and tripe might be due to higher pH[7,14] and collagen content[7]. Even though heart had higher pH, it exhibited a poor WHC which might be due to the type of muscle. Cooking release volume did not differ significantly among different meats but heart showed relatively higher cooking loss due to poor WHC. EC was significantly higher in head meat and thigh meat than others due to relatively higher salt soluble proteins[9,15].

From the results head meat ranked better than tripe and heart and is comparable with thigh meat while tripe meat was better than heart for inclusion in comminuted meat products. Detailed studies on the microbial quality and shelf life of these variety meats are suggested in view of their 'floor handling' practice which may result in greater contamination.

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BOOK REVIEWS

Diet-related Diseases, The Modern Epidemic—by S. Seely, D.L.T. Freed, G. A. Silverstone and V. Rippere. AVI Publishing Company Inc., West Port, Connecticut, 1985; pp: 272, Price: £ 9.95 in UK only.

This book consists of 9 chapters dealing with diet related diseases, mostly those that are seen in advanced countries.

In the first chapter, the authors have discussed briefly diseases known to be caused by the diet or due to failure in utilizing essential nutrients. Historical importance of diet-related epidemics and role of food as a source of infection have been touched upon.

Chapter 2 includes information on disorders of the circulatory system, cancer of the stomach, intestine and breast. Epidemiological evidence that these diseases could be of dietary origin has been presented. The attention of the reader has been drawn to some excellent books on the subject.

The known toxic effects of foods of plant and animal origin, microorganisms and food additives have been concisely, yet lucidly presented in Chapter 3.

The next chapter has covered diet related diseases of the arteries, theories on the onset of atherosclerosis and the role of cholesterol in the development of arterial diseases. Epidemiological data presented brings out the relationship between diet and coronary mortality. Special mention has been made on the atherogenic effect of milk.

Information on the geographical occurrence, dietary and/or hormonal causes of cancer of the GI tract, liver, breast and prostate has been presented in Chapters 5 and 6. Currently favoured theories on the subject, association of high glucose consumption, levels of thyroxine, cadmium and zinc deficiency with certain forms of cancer have been discussed briefly. Case histories of specific food intolerances, diseases caused by food intolerance, successful diagnosis, treatment and prevention of these diseases have been described briefly under Chapter 7. Immunological mechanisms, and role of food allergy in food intolerance have also been dealt with.

Material reviewed in Chapter 8 brings out the fact that diet, although essential to life, can cause mental malfunction and neurological disorders of various types. Disorders caused by caffeine, nutrient deficiency, neurotoxins like triorthocresyl phosphates, inorganic mercury compounds, food allergy and addiction have been mentioned. Involvement of inborn errors of metabolism affecting dietary constituents, formation of neurotransmitters from dietary precursors and neurotoxic peptides formed from foods in the development of mental illness and other neurological disorders have been discussed. Some of the disturbances can be alleviated by withdrawal of the offending causative agent but others may cause permanent structural damage of the nervous system and some may even cause death. The author suggests that these diet-induced disorders are avoidable and future work should involve application of existing knowledge in medical and behavioural sciences.

In the final chapter, the author has tried to seek the relationship between diet and old age. Though old age is not strictly related to diet, the right type of diet can prolong life. After a particular stage however an increase in calorie intake may tend to shorten life.

The book is very informative. Presentation is so clear that most of the portions of the book can easily be understood even by lay people. It will be a useful publication to all those interested in Food Science.

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

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

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

3. Names of chemical compounds and not their formulae should be used in the text. Methods of sampling, number of replications and relevant statistical analyses should be indicated. Superscripts and subscripts should be legibly and carefully placed. Foot notes especially for text should be avoided as far as possible.

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

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

6. **Illustrations:** Graphs and other line drawings should be drawn in *Indian ink* on tracing paper or white drawing paper preferably art paper not bigger than 20 cm (oy axis) × 16 cm (ox axis). The lettering should be such that they are legible after reduction to column width. Photographs must be on glossy paper and must have good contrast; *three copies* should be sent.

7. Abbreviations of the titles of all scientific periodicals should strictly conform to those cited in the World List of Scientific Periodicals, Butterworths Scientific Publication, London, 1962.

8. **References:** Names of all the authors along with title of the paper should be cited completely in each reference. Abbreviations such as *et al.*, *ibid*, *idem* should be avoided.

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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 issue of the *Journal* for guidance.

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