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Research Notes

Improvements to the Traditional Edge Runner Process for Rice Flake Production

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Low yield of rice flakes in the traditional methods is attributed to losses by breakage and powdering in the edge-runner. Fitting a second idle roller to the edge-runner reduces the flaking time from 60 to 40 sec and also the breakage from 5 to 3% and consequently increases the yield from 65 to 67%. A small roller flaker was developed with an effective roller length of 10 cm and tested for performance at different roller diameters and different speeds. The optimal diameter, peripheral speed and hardness were found to be 30 cm., 650 cm/sec, and 500 BHN (Brinell hardness number), respectively. The power consumption and capacity of the small roller flaker per unit length is comparable to that of corn flaker (4.75 kg/hr, and 0.13 kw per cm length). A small roller flaker with the edge-runner will facilitate fine flaking of coarse flakes, and increase the yield to 70%.

Continuous mechanised processes for making rice flakes (*Avalakki, Poha*) have been described earlier[1-3]. Major quantity of production is from small scale sector and therefore, improvements to the traditional process were attempted and the results are recorded here.

It has been reported that the edge-runner causes very little damage to rice in the early stages of flaking but the damage becomes heavy in the final steps of making thin flakes[2]. To minimise this loss the possibility of fitting an additional idle-roller to the existing edge-runner machine was tried. A small roller flaker for flaking of coarse flakes obtained from edge-runner and matching in capacity with it has been designed and used as an adjunct to the edge-runner to obtain thinner flakes.

Materials and Methods

Fitting of additional idle roller: One more idle-roller similar in size to that of the idle-roller already available with the machine was fabricated and fitted to the edge-runner with suitable reinforcements and supports.

Development of a small roller flaker: A small roller flaker with necessary mechanical drive was developed with an effective roller width of 10 cm and was tested for performance at roller diameter of 15, 20.5, 28.5 (cast iron) and 27.5 cm (stainless steel cladded cast iron) and run at peripheral speeds of 600-700 cm/sec and tested for its capacity using 50 kg lots roasted paddy. All the rollers were made of cast iron, except one set (27.5 cm) which was given stainless steel lining. Hardness of the rollers was tested using "Asha-poldi impact hardness tester (Portable model)."

The performance of the above flaker was compared to that of the commercial corn flaker[3] whose roller diameter and peripheral velocity were 45 and 450 cm/sec respectively.

Results and Discussion

Improvement of edge-runner: The reason for low yield of rice flakes in the traditional methods is attributed to breakage and powdering losses in the edge-runner, especially during the final stages of flaking that is between 30 and 60 sec. Quality and yield of rice flakes can be increased and breakage can be minimised if flaking time could be reduced by suitable modification of the machinery.

Increasing the speed of edge-runner or the weight of idle roller or giving postitive drive to the idle-roller was not found effective in reducing the flaking time.

Provision of a second idle-roller to the edge-runner was found to reduce the flaking time from 60 to 40 sec, reduce the breakage from 5 to 3 per cent and thus increase the yield of rice flakes from 65 to 67 per cent (Table 1). This may be due to the prevention of cooling and drying of grain[2]. It was found that paddy moisture content just before flaking should be less than that in the customary traditional process and should be about 22 per cent (dry basis). This could be obtained by soaking paddy overnight in water at initial temperature of 60°C, roasting the soaked paddy at 240-260°C and conditioning for 12-15 min between roasting and flaking. Higher moisture of about 24 per cent (dry basis) in the paddy caused lumping while lower moisture of about

TABLE 1. COMPARATIVE PERFORMANCE OF DIFFERENT FLAKING MACHINES

Parameters	Edge-runner			Small roller flaker[2]	Corn flaker[3]
	One roller	Two rollers	Small roller flaker[1]		
Head flakes (%)	65.0	67.5	70.0	68.5	68.7
Brokens (%)	5.5 (2.5)	4.5 (1.5)	3.1	2.5	2.5
Husk (%)	23.0	23.0	23.0	23.0	23.0
Degree of polish (%)	—	—	—	3.5	3.5
Flake powder (%)	—	—	—	1.7	1.8
Bran+flake powder (%)	5.8	4.4	4.0	—	—
Flaking time (sec,)	~50	~30	~25	Instant-aneous	Instant-aneous
Tempering time, (min)	4-6	12-15	4-6	—	—
Capacity, (kg/hr)	75	110	110	4.8 *	4.9 *
Power required, (KW)	2.5	3.0	3.0	0.13*	0.12*

Figures in the parenthesis indicate the flake powder lost with husk fraction

*Capacity and power required are computed per cm length of the roller.

1. Increase in yield is due to reduction in polishings.
2. Shelled and polished rice flaked in small roller flaker.
3. Shelled and polished rice flaked in corn flaker.

16 per cent (dry basis) caused breakage. The objective was to provide optimum conditions for the grain to be thermoplastic without being too dry or too lumpy. Grain moisture of about 22 per cent (dry basis) and temperature of 60°C were optimum. Rice obtained from roasted paddy could also be flaked in the modified edge-runner and brokens were free from husk fragments unlike in the customary process.

Development of a small roller flaker: The rollers with diameters of 15, 20.5 and 27.5 cm had an output of 40, 40 and 45 kg/hr whereas the roller with 28.5 cm diameter gave output of 50 kg/hr matching in capacity with that of edge-runner. The rollers of diameter 15 and 20.5 cm posed problem of nipping resulting in poor flaking. The capacity per unit length of this flaker is comparable to that of an imported commercial heavy duty roller flaker (45 cm diameter) and was about 4.8 kg/hr/cm. (Table 2)

It was also observed that smaller the roller diameter faster the rotation required for flaking. At higher or lower speeds, though the flaking was good the total output was reduced. At higher speeds the feed builds up in the roll presumably due to the reduction in coefficient of friction, which decreases the grip on the material.[4] The optimal peripheral speed of the rollers was found to be 650 cm/sec. Grain impressions were noticed on the cast iron rollers and stainless steel lined rollers as the

TABLE 2. COMPARATIVE PERFORMANCE OF HEAVY DUTY ROLLER FLAKER AND SMALL ROLLER FLAKER

Parameters	Heavy duty roller flaker	Small roller flaker			
Rolier variability					
Roller diameter (cm)	45	28.5	27.5	20.5	15.0
Roller hardness (BHN)	500	220	250	220	220
Optimum roller speed (r.p.m.)	200	400	500	850	1000
Capacity (kg/hr/cm width of roller)	5	4.8	4.5	4	4
Roller performance					
Yield of head flakes (%)	71	71	70	68	66
Breakage (%)	1.5	1.5	2.0	2.5	3.0

Brinell hardness number (BHN) was low-220 and 250, respectively as compared with 500 for the corn flaker rollers. It was found that throughput and power consumption were found to be about 4.75 kg/hr and 0.13 KW per cm width respectively which compares well with that of corn flaker. In view of this it is envisaged that the rollers made of chilled cast iron or alloy steel casting of 500 BHN and of 30 cm diameter roller and running at about 400 rpm would be suitable for this purpose.

The small roller flaker could be used for fine flaking of coarse flakes obtained from edge-runner and was similar to the commercial corn flaker reported by us[2]. This combined process eliminates the need for separate sheller and polisher and help in modernizing the existing mills manufacturing rice flakes without higher additional investment.

It was found that the processing cost for the (*i*) traditional process, (*ii*) traditional process with two idle rollers, (*iii*) traditional process with roller flaker as adjunct, and (*iv*) continuous process worked out to 42, 35, 37, 28 paise/kg of flake produced, respectively.

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Studies on the Utilisation of Kinnow and Malta Oranges

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Four commercial varieties of Malta namely 'Blood Red', 'Pineapple', 'Jafa' and 'Valencia Late' and one of 'Kinnow' orange, a mandarin hybrid were analysed for physico-chemical composition and suitability for processing and waste utilisation. Recovery of juice, peel and pomace in Malta varieties ranged from 50.8 to 55.4, 23.2 to 30.9 and 13.6 to 22.1 per cent respectively whereas in Kinnow the corresponding values were 55.8, 26.8 and 17.4 per cent respectively. Ascorbic acid content of Malta orange juice ranged from 51.2 to 62.5 mg/100g of juice and in Kinnow orange juice it was 46.9 mg/100g. Essential oil content of fresh peel of Malta and Kinnow orange was found to be 2.0 and 1.0 per cent respectively. Pectin content of Malta and Kinnow was found to be 5.73 and 2.0 per cent respectively, on FWB. Squashes prepared from juices of Malta varieties and Kinnow orange, were fortified with ascorbic acid at the rate of 100 mg/100g of juice. Squashes and ready-to-serve beverage prepared from comminuted Malta orange beverage base and Malta orange juice concentrate were found quite acceptable for six months storage at room temperature (25-35°C). Malta orange juice concentrate showed considerable loss in ascorbic acid during 6 months storage at room temperature, however, at low temperature (5-8°C) loss was only 19.2 per cent. An increase in reducing sugars from 13.6 per cent at low temperature to 26.3 per cent at room temperature and decrease in non-reducing sugars from 29.6 to 23.8 per cent was also noticed at room temperature during storage.

Among the citrus fruits, Mandarin is grown in Punjab, Karnataka, Maharashtra and Assam states in India. Kinnow orange is the first generation hybrid of 'King' and 'Willow Leaf' mandarins, evolved at the University of California, Regional Fruit Station. Kinnow in Punjab is very popular because of its good qualities[1-2].

While considerable work has been done on the processing of Indian mandarins and sweet oranges[3-9], the published information on the utilisation of Kinnow orange is rather scanty[3]. Since the keeping quality of Kinnow is not very satisfactory (6 to 9 days at RT) it was felt necessary to study the economic feasibility of processing of these oranges.

The Malta orange (*Citrus sinensis* Osbeck) is produced in Punjab state to the extent of 60,000 tonnes of which 20 per cent are undersized, have irregular shape and superficial peel blemishes, which could be utilised to produce processed products. The present study was undertaken (*a*) to screen the important commercial varieties of Malta oranges for processing, and (*b*) to work out the processes for the utilization of the Malta oranges.

Materials and Methods

Physico-chemical composition on Malta oranges: Four commercial varieties of sweet oranges, viz., 'Blood Red', 'Pineapple', 'Valencia Late' and 'Jafa' and one of Kinnow orange procured from the Regional Research Station, Abohar, Punjab were analysed for physico-chemical composition by employing standard AOAC[10] methods. The colour was extracted by acetone (1:10) and read at 400-700 nm.

Malta orange beverage base: Fully ripe, sound fruits were selected, soaked in 1.0 per cent hydrochloric acid solution for 5 min, drained, and then washed in plain water. The orange were steamed and cut into pieces. The pieces were mixed with known quantity of dilute sugar syrup and blended and passed through a pulper having 0.8 mm mesh sieve. The extract is formulated by adding other ingredients in such a way that when three tea spoons (20 g) of it is mixed with three tea spoons of sugar (26.5 g) and 150 ml of chilled water two tumblers of ready-to-serve (RTS) drink was obtained. The formulated base was filled in bottles, crown corked, and stored. The beverage was analysed for brix, acidity, viscosity etc. and also tested for organoleptic quality.

Malta orange juice concentrate: Washed fruits were cut into halves and juice extracted. The juice was passed through junior APV pulper having 0.8 mm mesh sieve and immediately pasteurised in plate type heat

exchanger at 90°C. It was centrifuged in a basket centrifuge at 3000 rpm to reduce the suspended solids to about 1 per cent. Then it was concentrated in a forced circulation evaporator to 62°brix. A portion of the concentrate was added with 1000 ppm SO_2 and filled in HDPE jerry cans. Another portion was canned in AR lacquered jam size cans and cold stored. Malta orange juice was stored at room temperature (25-35°C) and at 5-8°C.

Pure juice: Juice was extracted from Malta and Kinnow orange and analysed for important physico-chemical characteristics. Juices were preserved, with sulphur dioxide (350 ppm). One lot was pasteurised in bottles and another in plain tin cans for comparative studies.

Squashes: Squashes were prepared from both juices extracted from Malta and Kinnow oranges with juice: 25 per cent, brix 45°, acidity, 1.2 per cent (as citric acid) and SO_2, 350 ppm. Orange flavour and permitted food colour were added. Some samples were fortified with ascorbic acid (100 mg/100 g) bottled, crown corked and stored at room temperature (25-35°C) and at 5-8°C along with unfortified lots. Squash was prepared from comminuted Malta oranges also.

By-products from Kinnow and Malta oranges: Essential oil and pectin were extracted from fresh peels of Malta oranges as well as Kinnow oranges. Essential oil was determined using steam distillation by Clevenger's apparatus. Pectin was extracted by keeping the peel water ratio to 1:3 containing 0.25 per cent HCl and heating the mixture to 85-90°C for one hour after which the the mixture was cooled, strained and filtered through using supercel and filter paper pulp. The clear filtrate (pectin extract) was subjected to two volumes of alcohol and washed with different strengths of alcohol (70, 80, 90 per cent and absolute). The

TABLE 1. PHYSICAL CHARACTERISTICS OF MALTA AND KINNOW ORANGES

Physical characteristics	Fruit grade/size	Blood Red	Pineapple	Jafa	Valencia Late	Kinnow
No. of oranges/ ton	Small	6600	5600	5900	7900	6400
	Medium	5200	3900	4600	5400	5200
	Large	4500	3200	3900	3800	4500
	Average	5400	4200	4800	3700	5400
Mean wt/fruit (g)	Small	151.5	178.5	169.5	126.6	156.2
	Medium	192.3	256.4	217.4	185.2	192.3
	Large	222.2	312.5	258.4	263.2	222.2
	Average	188.7	249.1	214.4	191.7	190.2
Fruit height (cm)	Small	6.12	6.65	6.49	5.92	5.87
	Medium	6.77	7.40	7.11	7.00	6.41
	Large	7.13	8.31	8.45	7.57	6.67
	Average	6.67	7.45	7.35	6.83	6.32
Fruit diameter (cm)	Small	6.58	6.74	6.62	6.11	6.65
	Medium	7.44	7.61	7.10	7.03	7.44
	Large	7.58	8.34	8.85	7.91	7.93
	Average	7.20	7.56	7.52	7.02	7.34
Juice recovery (%)	Small	50.7	49.9	52.3	55.1	59.3
	Medium	61.5	58.3	50.9	58.3	55.1
	Large	54.1	44.2	57.3	51.7	53.1
	Average	55.4	50.8	53.5	55.0	55.8
Peel (%)	Small	30.0	28.2	28.2	20.6	25.5
	Medium	30.8	27.1	27.7	22.5	26.0
	Large	31.8	28.1	31.2	26.6	28.8
	Average	30.9	27.8	29.3	23.2	26.8
Pomace (%)	Small	19.3	22.9	19.5	24.3	15.2
	Medium	7.7	14.6	22.0	19.2	18.9
	Large	13.8	28.7	10.8	22.0	18.1
	Average	13.6	22.1	17.4	21.8	17.4

TABLE 2. SOME CHEMICAL CHARACTERISTICS OF MALTA AND KINNOW ORANGE JUICES

Varieties	°Brix	Acidity (as citric %)	°Brix/acid ratio	Ascorbic acid (mg/100 g)	Sugars			Viscosity (centipoises)
					Reducing	Non-reducing	Total	
Blood Red	10.0	0.60	16.6:1	62.5	4.6	1.6	6.3	8.0
Pineapple	9.5	0.58	16.4:1	53.1	3.9	0.3	4.2	7.0
Jaffa	10.0	0.64	15.6:1	51.2	3.5	0.3	4.9	8.0
Valencia Late	10.0	0.70	14.3:1	59.4	3.6	0 4	4.0	7.0
Kinnow	10.0	0.70	13.0:1	46.9	2.2	0.8	3.1	8.0

pectin was dried at 60°C overnight. Peel and pomace of Kinnow orange and four varieties of Malta oranges were cut into small pieces, blanched in simmering water (10 min for peel and 3-5 min for pomace), sun-dried and stored for later recovery of pectin and essential oil.

Results and Discussion

Physico-chemical composition of Malta oranges: Data on diameter, height of fruits and number of fruits per kilogram were also collected. Average weight per fruit of Malta oranges ranged from 188.7 ('Blood Red') to 249.1 g ('Pineapple'), however the mean weight of Kinnow orange was 190.2 g (Table 1). The recovery of juice was 55.4, 50.8, 53.5, 55.0 and 55.8 per cent in 'Blood Red' 'Pineapple', 'Jafa', 'Valencia Late' and Kinnow Orange respectively. Pomace content was the lowest in 'Blood Red' orange and highest in 'Pineapple' variety while peel content was the lowest in 'Valencia Late' and highest in 'Blood Red' orange.

Juice: Data on chemical composition of juices (Table 2) show that there was not much difference in brix in Malta varieties as the range was only from 9.3 to 10.0, acidity ranged from 0.58 to 0.70 per cent and ascorbic acid from 51.2 to 62.5 mg/100 g. Variation for viscosity was from 7 to 8 centipoises, reducing sugars 3.5 to 4.6 per cent and total sugars 4.2 to 6.3 per cent. Brix, acidity (%), ascorbic acid (mg/100 g), viscosity, reducing sugars and total sugars in Kinnow orange were found to be 10.0, 0.70, 46.9, 8.0, 2.2 and 3.1, respectively (Table 2). Thus, ascorbic acid content was lower in Kinnow orange than in any of the Malta varieties. Juice from Malta and Kinnow orange were preserved well with SO_2 (350 ppm) upto six months at room temperature but later on they developed slight bitterness. Colour also faded slightly but it maintained a good flavour.

Essential oil in peel: Essential oil content (per cent) of fresh Malta and Kinnow orange peel was found to be 2.0 and 0.75 to 1.0 respectively. However, in sun-dried peels of 4 varieties of Malta and Kinnow orange it varied from 2.2 to 2.5 per cent (V/W).

Pectin in peel and pomace: Pectin content of fresh Kinnow orange peel was 2.0 per cent on fresh weight basis (FWB) and 9.2 on dry weight basis (DWB). Pectin content of Malta oranges in fresh peel was 5.73 per cent on FWB and 23.1 per cent on DWB. In pomace of Malta oranges, pectin was found to be 2.3 per cent on FWB and 8.6 per cent on DWB.

Squashes and RTS beverages: Half of the lots of squashes prepared from Malta and Kinnow orange juice were fortified with ascorbic acid (100 mg/100 g juice) while the other lots were kept as control. The initial physico-chemical and organoleptic qualities of Malta orange and Kinnow orange squashes were recorded (Table 3). Ready-to-serve beverage was prepared from comminuted Malta orange beverage-base and Malta orange juice concentrate. Brix, acidity and viscosity of RTS beverage were found to be 15°, 0.23 per cent and 5 centipoises, respectively. Malta and Kinnow orange products remained in very good condition upto six months storage at room temperature. Organoleptic evaluation of RTS beverages of both the oranges made by a panel of judges found quite satisfactory upto six months of storage at room temperature. The colour of the beverages kept at low temperature remained unchanged throughout the experiment while those kept at room temperature slightly turned brown during sixth months. But this brown discolouration did not appear in those fortified with ascorbic acid. Taste and flavour were satisfactory upto 3 months storage but decreased slightly during six months storage. But in overall evaluation products were quite satisfactory.

Comminuted Malta orange beverage base: Comminuted Malta oranges beverage base had a brix of 12°, acidity 2.0 per cent and viscosity 180 centipoise. Coloured and flavoured beverage base scored more (31.1 out of 40) than did natural/uncoloured beverage

TABLE 3. PHYSICO-CHEMICAL CHANGES DURING STORAGE OF KINNOW ORANGE AND MALTA ORANGE SQUASHES

	°Brix	Acidity (%) (W/W)	Vit. C (mg/100) As it is	Vit. C (mg/100) Fortified	SO_2 (ppm)
			Initial		
Kinnow orange	45.0	1.2	46.9	105.9	356
Blood Red	45.0	1.3	62.5	108.9	359
Jafa	45.5	1.1	51.2	106.3	353
Pineapple	45.5	1.3	53.1	108.2	356
Valencia Late	45.0	1.2	59.4	107.8	353
			After 3 months at 25-35°C		
Kinnow orange	45	1.2	23.2	51.2	320
Blood Red	45	1.3	30.5	52.8	320
Jafa	45	1.2	23.8	49.5	304
Pineapple	45	1.2	25.9	51.2	320
Valencia Late	45	1.2	29.3	52.8	304
			After 3 months at 5-8°C		
Kinnow orange	45	1.2	31.8	59.4	304
Blood red	45	1.3	39.6	61.1	320
Jafa	45	1.2	33.5	59.4	304
Pineapple	45	1.3	34.7	62.7	304
Valencia Late	45	1.2	38.1	62.7	304
			After 6 months at 25-35°C		
Kinnow orange	45	1.2	19.4	42.5	307
Blood red	45	1.3	25.3	43.7	304
Jafa	45	1.2	19.2	41.8	301
Pineapple	45	1.2	21.3	42.1	307
Valencia Late	45	1.2	25.0	42.8	304
			After 6 months at 5-8°C		
Kinnow orange	45	1.2	24.6	49.7	307
Blood red	45	1.3	32.3	52.0	310
Jafa	45	1.2	36.5	49.8	307
Pineapple	45	1.2	27.4	53.1	304
Valencia Late	45	1.2	31.0	53.0	300

base (27.1). It remained in excellent condition upto six months storage at room temperature (25-35°C). Brix, acidity and viscosity of RTS beverage prepared from these comminuted Malta orange base were 15°, 0.23 per cent and 5 centipoises, respectively.

Comminuted orange squash: It retained excellent colour and flavour during 6 months' storage at room temperature (25-35°C).

Storage studies on Malta orange juice concentrate: During 6 months' storage at room temperature, considerable loss in ascorbic acid (about 90 per cent) was noticed in Malta juice concentrate but only 19.2 per cent loss in ascorbic acid was found in the concentrate kept at low temperature. There was an increase in reducing sugars by 26.3 per cent at room temperature and 13.6 per cent at low temperature while total sugars remained constant. Non-reducing sugars decreased from 29.6 to 23.8 per cent at room temperature and to 26.2 per cent at low temperature (Table 4). No change in acidity was observed. Changes in brix were insignificant. Changes in colour as seen from absorption spectra showed considerable darkening of concentrate kept at room temperature while the colour of concentrate kept at low temperature remained practically unchanged (Fig. 1).

TABLE 4. PHYSICO-CHEMICAL CHANGES IN MALTA ORANGE JUICE CONCENTRATE AFTER SIX MONTHS STORAGE AT ROOM TEMPERATURE AND AT LOW TEMPERATURE

Chemical characteristics	Initial	Storage for 6 months at 25-35°C	Storage for 6 months at 5-8°C
°Brix	73.6	72.0	73.1
Acidity (as % citric)	3.6	3.6	3.6
Assorbic acid (mg/100g	308.0	30.0	248.0
Reducing sugars (%)	24.3	30.7	28.1
Total sugars (%)	57.2	57.0	57.3
Non-reducing sugars (%)	29.6	23.8	26.2
Viscosity (centipoises)	3900	3700	3800

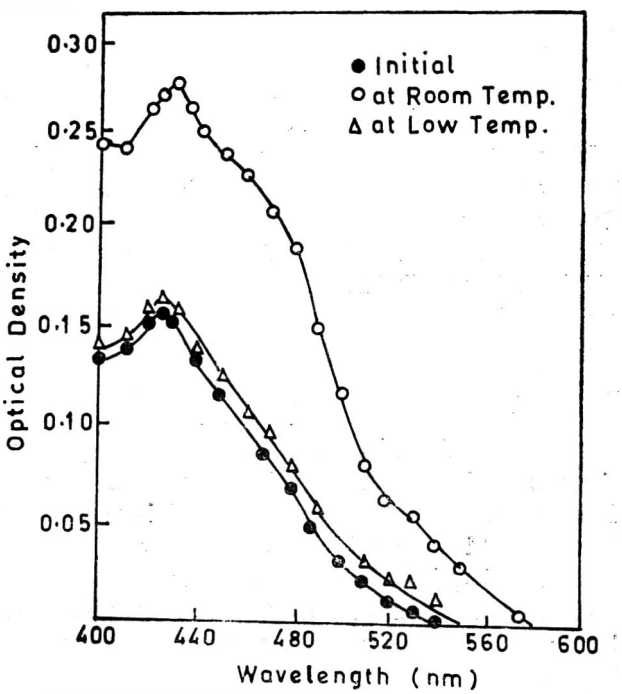

Fig. 1. Changes in visible spectra of Malta orange juice concentrate (acetone extract 1:10) after 6 months storage)

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Etiology of Soft Rot in Stored Potato

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Distribution of various microorganisms was examined on healthy potatoes of indigenous cultivars. It was observed that the tubers harbour Gram positive bacteria belonging to the genera *Bacillus* and *Micrococcus* along with Gram negative pectolytic cocobacilli, *Pseudomonas* and *Erwinia*. However major etiological agent of the soft rot in stored (above 15°C) potatoes was found to be *Erwinia carotovora*. This organism was located in the lenticels and vascular region. The losses in the tubers stored below 10°C could not be attributed to these organisms.

Among major factors causing losses in fruits and vegetables is the microbial spoilage[1]. *Erwinia*, *Bacillus* *Pseudomonas*, *Clostridium* and *Flavobacterium* spp. are normally associated with decaying vegetables[2]. Blackleg, a major field disease of potato is caused by *Erwinia carotovora* var. *atroseptica* (Van Hall) Dye[3]. This pathogen along with *E. carotovora* var *carotovora* (Jones) Dye, has been shown to produce soft rot of the tubers in field as well as during storage[3]. Blackleg is not a major problem in India though soft rot prevails[4,5]. Therefore, the microflora of healthy tubers of the indegenous cultivars was examined. The predominant pathogens causing spoilage of the tubers were identified and the responses of these organisms to some physicochemical treatments were ascertained.

Materials and Methods

The source of potatoes: Mature and cured tubers of cultivars, 'Kufri Chandramukhi', 'Kufri Kuber', 'Kufri Sheetman' and 'Kufri Sindhuri' grown at Simla were procured from the Central Potato Research Institute, Simla.

Periderm of five unwashed, medium sized, healthy tubers was scraped, from which 2 g was homogenized in sterile distilled water in sterilized omnimixer for two min and final volume was adjusted to 20 ml. Peri-

derm homogenates in triplicates were prepared likewise from the tubers of different cultivars and plated alter appropriate dilutions, on various growth media (Difco).

For estimating total bacterial and fungal counts, nutrients agar, pH 7.2 and potato dextrose agar, pH 5.6 respectively, were employed[6]. MacConkey agar, pH 7.1 was used to estimate Gram –ve bacteria[6]. Stewart's medium[7] was used to enumerate total pectolytic bacteria as well as to distinguish between *Pseudomonas* and *Erwinia* sp. The former developed white or yellowish colonies with shallow cavities around while the latter formed deeply sunk pink or reddish colonies. The suspensions were surface seeded and colonies were examined and counted after 36h.

Enumeration of erwinias: Tubers ('Kufri Chandramukhi') were washed in running tap water and air dried before tissue samples from various regions were obtained. From every tuber, ten lenticels were scooped out with a needle and ground in sterile distilled water in a pre-sterilized (with ethanol) mortar. Final volume of the suspension was adjusted to 1 ml. The cylindrical plugs were asceptically drawn perpendicular to the long axis of the tuber with a cork borer (1 cm diameter). Small discs (1 mm thickness) were obtained from the vascular and cortical regions. The discs thus obtained were ground (1 g in total volume of 20 ml) in a mortar. In all, 25 tubers were used, each for lenticels and internal tissue sampling. Suspensions were plated on Stewart's medium as described above. All biochemical tests performed to identify bacterial isolates were as described by Dye[8–10].

Washing of the tubers: The tubers ('Kufri Chandramukhi') were dipped in tap water in a stainless steel kettle for 15 min. After draining the water the dipping procedure was repeated once more. The tubers were then air dried on filter paper sheets.

Chemical or hot water dip: About 100 washed potatoes were dipped in 100 l. of hypochlorite solution (1000 or 2000 ppm available chlorine) or salicylic acid solution (10 per cent w/v) for 30 min. Similarly, washed tubers were dipped in hot water (52°C±3 for 10 min or 58°C±3 for 5 min) in a steam jacketed kettle. After treatments, the tubers were air dried and stored in jute bags at 30°C (90 per cent RH). At the end of 4, 8 and 12 weeks healthy tubers were counted.

Influence of environmental conditions on rotting: Seven lots of 150 tubers ('Kufri Chandramukhi') each, were made. Each lot consisted three replicates of 50 tubers. All lots were stored at 30°C (RH 90 per cent). The tubers were stored under the following seven sets of conditions to ascertain the influence of individual predisposive factors relating to water status, oxygen availability and mechanical damage. (*i*) Dry, unwashed tubers kept in jute bags; (*ii*) tubers wetted by dipping in water for 5 min and then transferred without air drying to jute bags; (*iii*) undamaged sound tubers kept in a single layer in trays; (*iv*) undamaged sound tubers stored in polythene bags (400 guage); (*v*) damaged tubers kept in a single layer in trays; (*vi*) damaged tubers kept in polyethene bags; and (*vii*) damaged tubers wetted as in (*ii*) and kept in polythene bags.

The tubers which were kept in jute bags and stored at different temperatures (2, 10, 15 and 30°C) were examined weekly for soft rot lesion. Those showing lesions were analysed microbiologically. From the advancing edge of the soft rot, tissue was macerated, suspended in sterile, distilled water and plated on Stewart's medium[3]. Three representative colonies per plate per tuber were picked, purified and identified. The tissue suspensions after holding for 10 min at 80°C were also inoculated in test tubes containing cooked meat medium (pH 7.2)[6] as well as in tubes containing pectate broth followed by anaerobic (with a layer of sterile liquid paraffin) incubation at 30°C for 15-20 days.

Results

Microbiology of healthy tubers: Table 1 shows that potatoes used in these studies were highly contaminated. The harbouring microflora comprised a large population of Gram positive bacteria, mainly *Bacillus*, *Micrococcus* and *Sarcina* sp. Appreciable number of fungal contaminants largely comprising *Aspergillus* sp. were detected. In addition, about 10^3 erwinias and 10^2

TABLE 1. NORMAL MICROFLORA OF TUBER SURFACE

	Total bacterial count	Total fungal count	Total Gram -ve bacteria	Pectolytic *Pseudomonas* count	Pectolytic *Erwinia* count
Kufri Chandramukhi	$1.2–4.3\times10^7$	$1.1–3.6\times10^2$	$1.6–4.2\times10^5$	$2.1–7.3\times10^1$	$3.0–8.1\times10^3$
Kufri Alankar	$4.3–7.6\times10^7$	$1.7–2.8\times10^3$	$2.8–5.1\times10^4$	$2.1–7.8\times10^1$	$3.3–5.5\times10^3$
Kufri Kuber	$2.9–3.9\times10^7$	$0.7–2.1\times10^2$	$5.1–9.3\times10^5$	$1.2–4.3\times10^2$	$5.1–8.5\times10^3$
Kufri Sheetman	$2.8–5.8\times10^6$	$4.6–9.0\times10^1$	$1.6–6.8\times10^4$	$0.6–3.1\times10^1$	$5.5–7.0\times10^3$

TABLE 2. ENUMERATION OF VARIETIES OF GROUP *CAROTOVORA*

Cultivar	Var. *carotovora*	Var. *atroseptica*	Intermediate strains
Kufri Chandramukhi	25.0	62.5	12.5
Kufri Kuber	22.5	52.5	25.0
Kufri Alankar	30.0	57.5	12.5
Kufri Sheetman	32.5	60.0	7.5

The number expresses percentage incidence of individual variety on tuber surface. Forty erwinial isolates per cultivar were identified and classified.

pseudomonas (per g of periderm) were present. Classification of erwinial isolates into the groups and varieties as shown in Table 2 indicated that incidence of *E. carotovora* var. *atroseptica* was much higher (upto 62 per cent) as compared to the of *E. carotovora* var. *carotovora* (upto 32 per cent).

Studies on distribution of bacteria inside the tubers revealed that cortical region of all the tubers examined was devoid of any microflora. On the other hand, tissue slices from vascular region of 15 tubers were found to contain 60-120 erwinias per g, while in 10 tubers, this region was devoid of any organisms. Sampling of lenticels indicated presence of erwinias in all the tubers examined. These bacteria were present in the range of 5-33 cells per lenticel.

Effect of physicochemical treatments: Washing the tubers in tap water and dipping in antibacterial solutions or hot water did not extend shelf life of the tubers. It is apparent from Table 3, that inspite of careful scrubbing of the tubers or dipping in antibacterial solutions, there still remained about 10^2-10^3 bacteria, comprising mainly erwinias per g of the periderm. The lenticels of the surface sterilised tubers had almost similar erwinial counts as lenticels of untreated tubers.

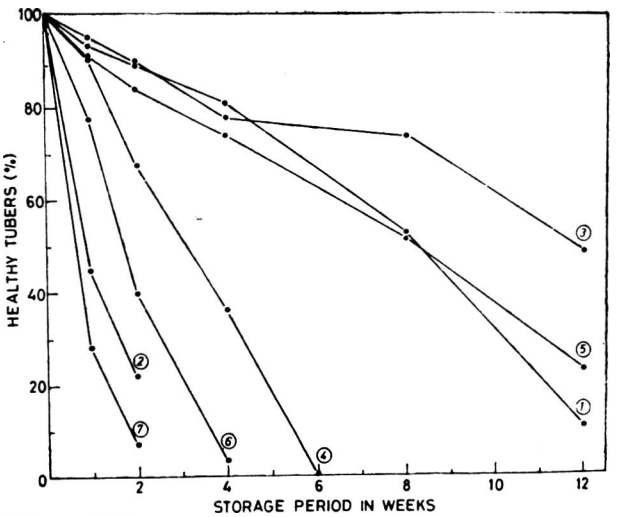

Fig. 1. Influence of moisture status, mechanical damage and oxygen availability on storability of tubers.

(1) Dry tubers in jute bags, (2) wetted tubers in jute bags, (3) undamaged tubers in open trays, (4) undamaged tubers in sealed polythene bags, (5) tubers showing signs of mechanical damage in open trays, (6) tubers showing signs of mechanical damage sealed in polythene bags, and (7) wetted, mecahnically damaged tubers sealed in polythene bags.

Factors influencing soft rot: As illustrated in Fig. 1 storage of wet tubers was most effective predisposive condition for the development of soft rot. It can be seen that 75 per cent of the wetted tubers spoiled in 15 days, whereas only 15 per cent of the dry tubers developed soft rot. Similarly, the tubers with mechanical injury and pathological blemishes spoiled much earlier than the sound tubers. Likewise, insufficient oxygen in sealed polythene bags (atmospheric oxygen present would be utilized by the respiring tubers thereby creating oxygen depletion) accelerated spoilage in both sound and damaged tubers. Combination of all the three conditions resulted in 100 per cent losses in a short span of two weeks.

Predominant pathogen of soft rot: As seen from Table 4, the tubers of all the cultivars stored at 15 and 30°C

TABLE 3. INFLUENCE OF PHYSICOCHEMICAL TREATMENTS ON MICROFLORA OF TUBER SURFACE

Treatment	Total bacterial count	Total fungal count	Total Gram -ve bacteria	Pectolytic *Pseudomonas* count	Pectolytic *Erwinia* count
Unwashed	$2.7\text{-}3.9\times10^7$	$4.0\text{-}6.0\times10^4$	$0.73\text{-}6.1\times10^4$	$6.0\text{-}7.2\times10^1$	$3.\ 0\text{-}4.0\times10^3$
washed	$6.0\text{-}8.0\times10^5$	$1.0\text{-}2.2\times10^3$	$2.3\text{-}6.0\times10^3$	8–30	$0.96\text{-}2.5\times10^3$
Washed and scrubbed	$4.3\text{-}5.7\times10^4$	0–5	$3.0\text{-}4.5\times10^2$	nil	$6.\ 0\text{-}8.3\times10^2$
Washed and dipped in hypochlorite (2000 ppm for 30 min)	$3.1\text{-}4.0\times10^3$	nil	16–100	nil	$1.0\text{-}5.0\times10^2$
Washed and dipped in hot water (52°C for 10 min)	$0.4\text{-}6.9\times10^3$	12–27	$3.1\text{-}5.6\times10^2$	6–30	$0.5\text{-}5.8\times10^2$

TABLE 4. PREDOMINANT SOFT ROT PATHOGENS OF STORED POTATO

Cultivar	Storage** temp. (°C)	Per cent incidence* of pathogens identified as					
		Bacillus	*Pseudomonas*	*E. C.* var *carotovora*	*E. C.* var *atroseptica*	*E. C.* var *chrysenthemi*	Intermediate strains of *Erwinia*
Kufri	15	ND	10	20	60	—	10
Chandramukhi	30	10	10	20	40	—	20
Kufri	15	—	10	30	50	—	10
Kuber	30	10	10	30	40	10	—
Kufri	15	—	—	20	60	—	20
Alankar	30	20	—	30	50	—	—
Kufri	15	—	10	20	60	—	10
Sheetman	30	10	—	30	40	10	10
Kufri	15	—	—	20	50	—	30
Sindhuri	30	10	—	40	40	10	—

*Twenty isolates per temperature per cultivar were identified.

**Tubers stored at 10°C (or below) did not show soft rot symptoms.

ND Not detected.

were spoiled by *E. carotovora* var. *atroseptica*. This pathogen could be isolated from about 50 per cent of the rotting tubers. *Pseudomonas* and *Bacillus* sp. though present on healthy tuber in large numbers were not major pathogens of spoilage. Anaerobically incubated cooked meat and pectate broth media (which were inoculated with heated rotting tissue suspensions) showed no sign of bacterial growth. This excluded the possibility of *Clostridium* sp. being soft rot pathogen. In the tubers stored below 10°C, soft rot development was not evident Nevertheless, a small proportion (about 5 per cent) of tubers developed dry rot. A non-pectolytic bacterium identified as *Micrococcus* sp. was found to be associated with the rotting tissue.

The causative agents were found to be the same, whether the tubers were spoiled naturally over a period of 2-3 months or under artificial conditions created to accelerate soft rot development (in 3-4 days) as indicated above.

Discussion

The microbial load on healthy tubers presumably arising from the adhering soil, largely contained Gram +ve bacteria. However, this is of little significance in relation to soft rot since these bacteria are not known to be the primary pathogens of this disease[2]. Similarly, the fungal flora on the tubers are not considered of consequence[2]. However, the pectolytic Gram -ve bacteria like *Pseudomonas* and *Erwinia* are known to cause the soft rot[2]. Latent infection of the tubers by these pectolytic bacteria has been reported[11]. Tubers were found to be contaminated by fungi like *Aspergillus* and *Penicillium* sp., however none of these fungal isolates were responsible for soft rot during storage.

Many pectate based selective media have been recommended for enumeration and isolation of pectolytic bacteria from the diseased plant tissue and soil. The suitability of Stewart's medium[7], Logan's medium[12] modified Stewart's and Logan's media[13], Cuppel and Kelman's crystal violet pectate medium[14] and Paton's medium[15] was examined by using the standard cultures of *E. carotovora*, *Pseudomonas fluorescens* and *Bacillus* sp. Among these media, modified Stewart's and Logan's media (without bile salts added) were found to have a disadvantage of permitting the growth of Gram +ve bacteria. Logan's and Paton's media failed to distinguish between pseudomonads and erwinias, whereas Cuppel and Kelman's medium was observed to suppress to a certain extent the growth of erwinias. The medium recommended by Stewart was found to be the most suitable since these limitations were not noticed. This medium was used throughout the studies for selective enumeration and isolation of pectolytic erwinias and pseudomonads.

In present studies, *E. carotovora* var. *atroseptica* was found to be the predominant soft rot pathogen. It is noteworthy that inspite of negligible incidence of blackleg in India, the pathogen has been found to occur on

healthy tubers and to cause soft rot during storage. Survival of this pathogen on the tubers throughout the storage period has been known[3]. These pathogens do not survive freely in soil, but remain alive in association with either healthy or rotting vegetative tissue. Also, the mother tuber carrying these pathogens could serve as a source of infection, thus the tubers of plants free of blackleg too could harbour the pathogens[3], The low incidence of blackleg disease in India could be due to unfavourable conditions of environment.

The genus *Erwinia* comprises both, pectolytic and non-pectolytic members[8-10]. Three major groups have been indicated: (*a*) *Amylovora*, responsible for dry necrosis, galls or wilts of plants, (*b*) *Herbicola* comprising yellow pigmented saprophytes associated with plants, and (*c*) *Carotovora*, constituting the soft rot pathogens, namely var. *Carotovora*, var. *atroseptica* and var. *Chrysenthemi*. In the present investigations, all these three varieties were found to be associated with the rotting potatoes stored at temperatures of 15 and 30°C. At lower temperatures however, soft rot was not evident. The factors responsible for the absence of soft rot at 10°C could be lowered growth rates and endopectate lyase levels in pathogens as well as increased diseases resistance of potato by way of accumulation of high levels of phenolics and phytoalexins at this temperature[16]. *Carotovora* group differs from all other bacterial genera in having strong pectolytic property. Therefore, a separate genus, *Pectobacterium* has been suggested for this group by taxonomists[2].

In the present studies, about 15 per cent isolates of *Erwinia* exhibited mixed biochemical reactions. These isolates could not be placed in any of the sub-groups based on classification scheme recommended by Dye[8–10]. In view of their limited incidence, they were not considered of any significance. It has been suggested that intermediate strains could arise as a result of hybridization among the sub-groups[2].

The present studies have indicated that besides temperature, other environmental factors like low oxygen concentration in atmosphere and high water activity on the tuber surface could also accentuate soft rot. Apart from favouring microbial activity water film present on the tuber could also result in oxygen depletion in the host.

Apparent ineffectiveness of physicochemical treatments in extending the shelf life of the potaotes indicated that the potential pathogens are protected. This was confirmed when pectolytic erwinias were detected in lenticels and vascular region.

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Studies on Transportation of Anab-E-Shahi Grapes

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Effects of post-harvest treatment by fumigation with sulphur dioxide, and the inpackage fumigant on quality of 'Anab-e-shahi' grapes during transportation by rail from Hyderabad to Ludhiana and to Mysore were studied. Both treatments helped in preventing the losses due to berry drop and decay and in maintaining the fresh appearance of bunches. Inpackage fumigation has proved practical and more suitable as it is cheap and simple to adopt.

'Anab-e-shahi' is the main variety of grapes cultivated, in Andhra Pradesh which accounts for 90 per cent of the total production in the region. During the season, over 60 per cent of the produce is exported to distant markets like Delhi, Calcutta, Bombay, Madras and Coimbatore by train, which takes 2 to 3 days to reach the destination.

Considerable loss and spoilage of grapes due to berry drop and decay take place during handling and transport, ultimately affecting the quality and marketability of grapes. Earlier, Narasimham *et al*[1] reported the effect of pre-harvest spray of growth regulators and fungicides[2] like SOPP and Captan on the control of berry drop and decay in 'Anab-e-shahi' grapes. Rao *et al.*[3] also reported the effect of growth regulators on the berry drop. Anandaswamy *et al.*[4] have reported the effect of pre-harvest sprays with formulations containing both growth regulators and fungicide on the spoilage of 'Anab-e-Shahi' grapes packed in different packages during transportation from Hyderabad to Lucknow. Earlier studies carried out at this laboratory[5] indicated that grapes can be stored at room temperature in the Bamboo baskets for more than 7 days by fumigation of berries with SO_2 fumes before packing or by placing an inpackage fumigant in the middle of the basket, by which both the berry drop and decay were reduced. The present studies were conducted to assess the effect of these two simple techniques on the quality of grapes when transported in conventional baskets by rail to distant places.

Materials and Methods

A healthy grape orchard of 5 year old vines near Hyderabad was selected for the studies. Ripe grape bunches were harvested from the late season crop (i.e., in the last week of March) by commercial pluckers in conventional manner on the same day. Five kilograms of grapes of uniform maturity were packed in each of 120 bamboo baskets which are normally used by the trade for rail transporation. The basket is cylindrical in shape with 30 cm diameter at the top and 20 cm at the bottom and 25 cm depth and is provided with a circular foot rest, a concave covering lid and a handle to carry. The grape bunches are packed in the basket in three layers from bottom to the top with paper shred partition between each layer and at top and bottom. The basket is finally closed with bamboo lid and stitched.

The experiment was in randamised block design with three treatments viz., (*i*) Control—without any treatment, (*ii*) SO_2 fumigation of baskets after packing For this purpose 10 packed baskets at a time were placed in a sulphur fumigation chamber (1100 l volume) and were exposed to SO_2 fumes for 30 min by burning 8 g of sulphur (@ 0.6g for 100 l. volume). The SO_2 concentration in the chambers was maintained at 0.5 per cent V/V. (*iii*) Inpackage fumigation (IPF)—The grapes were packed into baskets as in control and a cloth bag of 5×5 cm size containing 5 g of potassium metabisulphite and 0.05 g of citric acid was placed in the middle of the basket in between two layers of the grape bunches. For each treatment 40 baskets were prepared.

Transportation to Ludhiana: From the above, 90 baskets (30 from each treatment) were transported to Ludhiana by express train on the same day. One transhipment of the consignment took place on the way at Delhi. Out of the baskets received in good condition, 3 baskets in each treatment were set aside at RT for observation 7 days after harvest. This was done

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to assess the quality of grapes in cases of unforeseen delays during transit and auction. Ten baskets from each treatment were sent to market on the 3rd day after harvest for auctioning to assess the consumer acceptability. The remaining baskets were opened in the laboratory to record the data on the general appearance, net weight, quantity of dropped and decayed berries.

Transportation to Mysore: Similarly, 30 baskets (10 from each treatment) were transported to Mysore by Express train on the same day. One transhipment of the consignment took place on the way at Bangalore. Since the number of baskets sent to Mysore were less, all the baskets were opened at Mysore on the third day after harvesting and recorded the data on the general appearance, PLW, dropped and decayed berries. No basket could be sent to market for assessing consumer acceptability.

The PLW, berry drop and decayed berries were recorded by physical and visible measurements. Organoleptic evaluation of the samples was done by a panel of five judges adopting a 5-point hedonic scale and the average was taken for the assessment of quality.

Results and Discussion

Transportation to Ludhiana: The consignment reached Ludhiana after 3 days. Out of the 90 baskets despatched, only 86 reached in good condition. The data on the condition of the consignment is given in Table 1.

Physiological loss in weight (PLW): The PLW of grapes 3 days after harvest in the control was more (3.9 per cent) than in the treated lots (2.5 and 2.6 per cent) and the difference is statistically significant.

Effect on mould growth, berry drop and decay: It is interesting to note that there was absolutely no mould growth in grapes packed with IPF, while the control and SO_2 fumigated grapes had only moderate mould growth. The decay of berries was highest in the control (16.3 per cent) and only moderate in the treated grapes (7.8-6.2 per cent), and the difference is significant. The berry drop was also highest in the control (14.2 per cent), while moderate in the treated lots (8.2 and 7.8 per cent), the difference being significant.

Effect on freshness of grapes and over all acceptability: The freshness of grapes is judged by the greenness of the peduncle and the rachis. In the freshly harvested grapes they are green, which turn brown on storage. The over all acceptability of grapes was highest in the case of IPF treated lots (4.1), moderate with SO_2 fumigated lot (3.7) and low in control (2.9). The overall high acceptability of the grapes with IPF treatment was due to the complete absence of mould attack, the retention of green colour and fresh appearance of the bunches.

Effect of storage at RT: The baskets stored at RT for 4 more days after receiving at Ludhiana i.e., total 7 days after harvest, were opened and examined. The results are given in Table 2. There is not much difference in the PLW of the three lots. However, there is marked improvement in the reduction of berry drop and decay in the IPF treated lot than the other two. It is also noteworthy that there was absolutely no mould growth in IPF treated lots as against moderate to heavy mould growth in the SO_2 fumigated and control lots. The overall acceptability of grapes in IPF lots is very high compared to the other lots over a period of 7 days storage at RT (30-35°C). The beneficial effect of IPF may be explained

TABLE 1. EFFECT OF DIFFERENT TREATMENTS ON THE QUALITY OF "ANAB-E-SHAHI" GRAPES DURING TRANSPORTATION TO LUDHIANA (3 DAYS) AND MYSORE (2 DAYS)

Treatment	Transported to Ludhiana					Transported to Mysore			
	PLW† (%)	Berry drop (%)	Decay (%)	Mould affected	Overall acceptability*	PLW† %	Berry drop %	Decay %	Mould affected
Control	3.9	14.2	16.3	Mod.	2.9	5.2	5.2	10.8	Neg.
SO_2 treated	2.5	8 2	7.8	Mod.	3.7	4.9	4.2	8.6	Neg.
Inpackage fumigation	2.6	7.8	6.2	Nil	4.1	4.6	3.9	2.9	Nil
S.Em	±0.3	±22.0	±66.4	Nil	±0.13	±21.7	±4.7	±20.2	—
CD at 5%	0.5	4.4	7.6	—	0.34	NS	NS	4.2	—
CD at 1%	0.7	NS	NS	—	0.46	NS	NS	5.8	—

†=Physiological loss in weight. *Based on 5-point Hedonic scale Mod.=Moderate, Neg.=Negligible, NS=Not significant.

TABLE 2. EFFECT OF DIFFERENT TREATMENTS ON THE QUALITY OF ANAB-E-SHAHI GRAPES AFTER 7 DAYS AT RT (30-35°C) AT LUDHIANA

Treatment	PLW (%)	Decay (%)	Berry drop (%)	Mould growth	Overall acceptability
Control	7.1	11.7	14.0	Heavy	1.3
SO_2 treated	6.3	7.1	14.7	Moderate	2.3
Inpackage fumigation	7.1	4.9	6.3	Nil	4.0

due to the slow release of SO_2 from the fumigant mixture on contact with the moisture released by transpiration of the grapes. This helps in preventing the mould growth and in retaining the fresh colour.

Effect on general consumer acceptability: In order to assess the consumer acceptability and the commercial value of treated grapes, unopened baskets, 6 in control, 10 each in SO_2 fumigated and IPF treatments were sent for auctioning to the whole sale market at Ludhiana. Similarly, after taking the observations in the laboratory, the grapes were repacked and sent to the market for auctioning. Each basket was individually auctioned after removing the lid and examining the top layer by the customers. It was observed that with respect to unopened baskets the average rate at which each basket was sold was Rs. 15.58 (Rs. 12.00–20.25) for control, Rs. 15.62 (Rs. 13.00–19.25) for SO_2 fumigated and Rs. 17.15 (Rs. 13.50–21.00) for the IPF lot. The corresponding rates for opened and repacked baskets, were Rs. 15.36 (Rs. 12.75–21.15), Rs. 16.92 (Rs. 15.00–19.75) and Rs. 19.12 (Rs. 16.00–23.25) respectively, the values in brackets being the range in each case. In both cases the treated lots fetched more price than control.

Transportation to Mysore: All the 30 baskets sent to Mysore were received at the destination after 2 days in good condition. They were immediately opened and examined. The data on the condition of the grapes is presented in Table 1. In this case there was not much difference in PLW in all the lots. The berry drop was slightly less (4.2 and 3.9 per cent) in the treated lots than in the control (5.2 per cent). However, with respect to decay of berries, the IPF treatment exerted very significant beneficiary effect in controlling the disorder than in the other two lots. The mould growth on bunches in the case of control and SO_2 fumigated lots was only negligible, while the IPF treated lot is completely free from moulds, thus in this case again the IPF treated grapes registered high general acceptability than the other lots.

The results of the above investigation have clearly shown that the spoilage in 'Anab-e-shahi' during transportation and storage can be controlled to a great extent by adopting a simple technique of placing an inpackage fumigant in the middle layer of grapes during packing. The cost of the bag and the chemical would be around 50 paise per basket of 5 kg grapes. The IPF can protect the grapes from decay and mould growth upto 7 days, besides retaining the freshness of the bunches for a long time which ensures good returns.

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Purification and Regulatory Properties of Phosphoenolpyruvate Carboxylase from Banana Fruits of Dwarf Cavendish *(Musa sapientum)* Variety

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Phosphoenolpyruvate carboxylase (PEPC) activity remained unchanged during ripening of banana. PEPC was purified 84-fold and its regulatory properties were studied. It required Mn^{2+} and free sulfhydryl group for activity. Km for phosphoenolpyruvate (PEP) was found to be 0.11 mM and Hill coefficient was 1.6. Succinic, malic, aconitic, and α-ketoglutaric acids inhibited the enzyme activity competitively and pyruvic acid inhibited non-competitively. D-glucose-6-phosphate, D-glucose-1-phosphate and DL-3-glycerophosphate activated the enzyme by decreasing the Km for PEP.

Phosphoenolpyruvate carboxylase / orthophosphate : oxaloacetate carboxylase (phosphorylating) E.C. 4. 1.31/ is involved in irreversible carboxylation of phosphoenolpyruvate (PEP) to form oxaloacetate (OAA). This reaction results in the synthesis of malate, when coupled to malate dehydrogenase. The enzyme is widely distributed in nature. It is found in many genera of bacteria[1,2] and most of the plants[3,4] but not in animal tissues, yeasts and fungi.

PEPC plays an important role in malate synthesis in plants and bacteria. This enzyme is subjected to various controls in both microbial and plant systems. In bacteria PEPC is activated by acetyl-CoA[1], fructose-1, 6-diphosphate[2]. PEPC in plant systems, however, is not affected by acetyl-CoA and fructose-1, 6-diphosphate[3] but activated by glycine and inhibited by organic acids.

PEPC of "Dwarf Cavendish" banana fruits was partially purified and some of its properties are reported. The possibility of PEPC being under the control of various metabolic intermediates and its role in the accumulation of malic acid during ripening of banana is discussed.

Materials and Methods

Freshly harvested mature banana fruits (both 'Dwarf Cavendish' and 'Robusta' varieties) were obtained from a local garden. These fruits ripened within 15-20 days when stored at 26°C.

Four bananas each from pre-climacteric, climacteric and post-climacteric stages of ripening, were selected, pulped individually and PEPC activity was determined. Two hundred grams of pulp was homogenised with 50 ml medium consisting of 0.33 M sucrose, 0.05M Tris, 0.01 M ethylenediamine tetra acetic acid (EDTA), 0.5 per cent polyvinylpyrrolidone (PVP) and 0.05 per cent cysteine in a pestle and mortar maintaining the pH at 7.0. The homogenate was diluted about five times with the buffer, filtered through cheese cloth and the filtrate was centrifuged at $10,000 \times G$ for 20 min. About 10 ml of the supernatant was dialysed against 2 l. of 0.02 M Tris-HCl buffer pH 7.2 containing 2 mM EDTA for 4 hr. and then assayed for activity. All enzyme studies were carried out using Tris-HCl buffer of pH 7.2.

Assay of the enzyme activity: PEPC activity was assayed by coupling the reaction with MDH and following the rate of oxidation of NADH spectrophotometrically at 340 nm[5]. One milliliter of the reaction medium prepared in Tris-HCl buffer contained (in μ moles) 100:$MnCl_2$, 1:$KHCO_3$, 10:NADH, 0.15: PEP, 1 and 1-5 mg supernatant protein. Ten units of MDH were added to the purified preparation. Specific activity was expressed as moles of PEP carboxylated/min/mg protein. PEPC activity was also measured by the incorporation of $KH^{14}CO_3$ (specific activity 3×10^5 cpm/m moles) into acid stable fraction. Further [^{14}C] malic acid formed in the reaction medium was also separated by paper chromatography and analysed for radioactivity. The spectrophotometric method, however, was used for routine estimations of enzyme activity.

Purification of the enzyme: The supernatant from six ripe banana fruits ('Cavendish' variety) obtained as above (about 400 ml) was brought to 80 per cent saturation with ammonium sulphate and allowed

to stand for 20 min. The precipitate was collected after centrifugation at 13,000 × G for 15 min and suspended in 0.02 M Tris-HCl buffer pH 7.2 containing 2 mM EDTA and dialysed against the same with two changes of buffer. The dialysed sample was stored at –20°C for 24 hr. After thawing, it was centrifuged at 10,000 × G for 10 min. The sediment was discarded as it had no enzyme activity. To the supernatant (100 ml), alcohol was added drop by drop at 0°C to bring it upto 33 per cent, stirred well and allowed to stand at 0°C for 20 min. The precipitate was sedimented by centrifugation at 13,000 × G for 15 min and suspended in 0.02 M Tris-HCl buffer, pH 7.2 and dialysed against the same (21) for 2 hr. Dialysis for a longer period would lead to the loss of activity. The dialysate was applied to 15 × 1.5 cm DEAE-cellulose column (fine mesh, exchange capacity 0.9 meq/g) pre-equilibrated with 0.02 M Tris-HCl buffer, pH 7.2. The column was washed successively with 80 ml of 0.02 M, 0.15 M and 0.2M Tris-HCl buffer, 4 ml fractions were collected and the active fractions were pooled. The purified preparation was devoid of phosphatase and malic enzyme activities but contained small amounts of malate dehydrogenase activity. Protein was estimated by the method of Lowry *et al*[6].

Results and Discussion

PEPC activity was found to be confined to the soluble cytoplasm of banana fruit pulp whereas in some plants it is attached to chloroplasts[7,8] and particulate[9] fraction. There was no significant change in the enzyme activity during ripening in either of the varieties.

To study the regulatory properties of PEPC, it was purified 84-fold from ripe banana fruit ('Dwarf Cavendish' variety). PEPC was eluted with 0.2 M Tris-HCl buffer, pH 7.2 (fractions 42-47). The results of enzyme purification are given in Table 1. The pH optimum was found to be 8.0 being similar to other plants[10] and bacteria[1,2]. The enzyme required Mn^{2+} for activity. Km for Mn^{2+} calculated from double reciprocal plots was found to be 0.04 mM. At 0.2 mM, the enzyme activity with Co^{2+} and Mg^{2+} was 37 and 26 per cent respectively of the activity obtained with Mn^{2+}. At 1 mM concentration, Zn^{2+}, Cu^{2+} and Hg^{2+} completely inhibited the enzyme activity. p-Chloromercuribenzoate at 0.5 mM, inhibited the enzyme activity by 75 per cent and this inhibition, was relieved by the addition of 5 mM dithiothreitol, indicating the requirement of sulfhydryl group for enzyme activity. Apparent Km for HCO^-_3 (0.08 mM) was found to be lower than endogenous levels which is a general feature of PEPC[11].

Saturation curve of banana PEPC with respect to PEP was sigmoidal (Fig. 1) and Hill coefficient was 1.5 (Fig. 2) which indicates the possibility of existence of more than one binding site for PEP and positive cooperativity. Apparent Km for PEP calculated from the Hill plot was 0.11 mM.

Many compounds were tested for their effect on PEPC activities. MDH, the second enzymic component of the assay system was not affected by the compounds tested. Effects of succinate, malate, aconitate, ∝-ketoglutarate and pyruvate on banana PEPC activity are given in Table 2. The inhibitions produced by these organic acids was in the range of 20-45 per cent. Malate inhibition of PEPC activity has been reported in other plant systems[11-13]. Not much information is available regarding the nature of organic acid inhibition of PEPC

TABLE 1. PURIFICATION OF PEPC FROM RIPE BANANA (DWARF CAVENDISH VARIETY) FRUIT PULP

Step	Total activity (units)	Volume (ml)	Total protein (mg)	Specific activity (units/mg protein)	Purification
Crude extract after centrifugation	57,600	550	960	60	1
Ammonium sulphate ppt	56,160	100	520	108	1.8
Alcohol ppt	51,840	35	160	324	5.4
DEAE-cellulose eluant	20,664	25	4.1	5040	84.0

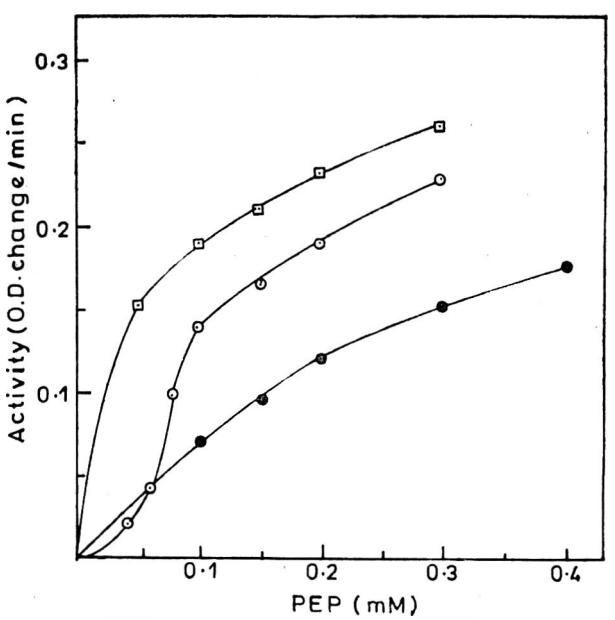

Fig. 1. Activation and inhibition of PEPC activity by glucose-6-phosphate (5 mM) and malic acid (5 mM) respectively. Control o——o; Glucose-6-phosphate, □——□; Malic acid ●——● . Assay conditions are same as described for kinetic studies in Table 2

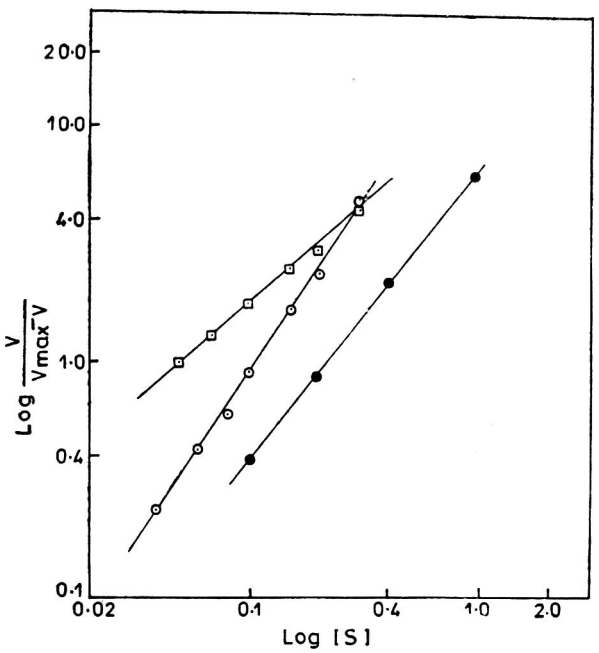

Fig. 2. Hillplots for PEPC activity in the presence of (1) Glucose-6-phosphate, □—□; Malic acid o—o; Control ●—●. Assay conditions are same as described for kinetic studies in Table 2.

activity. In the present investigation, the inhibition of banana PEPC activity by organic acids has been studied in detail. Malate, succinate, aconitate and ∝-ketoglutarate inhibited banana PEPC activity by decreasing the Km for PEP without changing V_{max}. The kinetics indicate that they are competitive inhibitors. Pyruvate was found to inhibit banana PEPC activity non-competitively. Km for PEP and n values in the presence of these acids are given in Table 2. Saturation curve and Hill plot as a function of PEP concentration in the presence of malic acid are shown in Figs, 1 and 2 respectively.

Aspartate at 10 mM concentration, inhibited the enzyme activity by 40 per cent when the concentration of PEP was 0.15 mM and this inhibition was not observed at saturating PEP concentration (5 mM). Fumarate, acetate, glutarate, glucuronate, glycine at 10 mM concentration and, FDP and acetyl-CoA at 3 mM concentration did not affect the enzyme activity significantly. The effect of these compounds was tested at two different concentrations of PEP which had given half maximal and maximal velocities. In bacterial systems[1,2] FPD and acetyl-CoA are known to activate the enzyme PEPC. These compounds, however, have been shown to have no effect on PEPC from higher plants[3].

ATP and ADP at 1 mM concentration, inhibited the enzyme activity by 40 and 25 per cent respectively. The inhibition was reversed by increasing Mn^{2+} concentration from 1 to 4 mM.

The effects of D-G-6-P, D-G-1-P and DL-β-glycerophosphate on PEPC activity are given in Table 3. These compounds activated the enzyme by decreasing the Km value without affecting V_{max}. Hill coefficient (Fig. 2) and K_m for PEP in the presence of these compounds are given in Table 3. Hill coefficient (n) decreased from 1.6 to 0.8 in the presence of these compounds. Activation of PEPC by G-6-P has been reported in higher plants[14]. G-1-P has been shown to have no effect on PEPC, in some plants[4].

Some fruits including banana are known to accumulate organic acids during ripening[15-17]. In banana, one of the acids to accumulate is malic acid which increases from 1.36 to 6.2 meq/100g fresh weight during ripe ning[15]. Enzymes involved in malic acid formation are malate synthetase,

TABLE 2. EFFECT OF ORGANIC ACIDS ON BANANA PEPC ACTIVITY

Organic acids	Concn (mM)	% inhibition*	Km for PEP** (mM)	Hill coefficient (n)
Succinate	3	56	0.46	0.75
Malate	5	45	0.23	1.10
Aconitate	5	37	0.37	0.94
∝-ketoglutarate	5	20	0.23	0.96
Pyruvate	5	36	0.12	1.20
Control	—	—	0.11	1.50

*PEP was added to the reaction medium at a concentration of 0.2 mM

**For kinetic studies, 4 mM $MnCl_2$ was used in the reaction medium and the concentration of PEP varied from 0.05 mM to 5 mM.

TABLE 3. ACTIVIATION OF BANANA PEPC BY SUGAR PHOSPHATES

Sugar phosphate*	% activation+	Km for PEP‡ (mM)	Hill coefficient (n)
D-Glucose-6-P	110	0.05	0.80
D-Glucose-1-P	80	0.06	0.79
DL-β-Glycero-P	120	0.05	0.82
Control	—	0.11	1.60

*The concentration of sugar phosphate in the assay medium was 5 mM.

+PEP was added to the assay medium at a concentration of 0.08 mM.

‡For kinetic studies, 4 mM $MnCl_2$ was used in the assay and the concentration of PEP varied from 0.02 to 5 mM.

malic enzyme, PEPCK and PEPC. Malate synthetase activity is known to decrease during ripening of banana[18] PEPCK, however, functions mostly in the formation of PEP from OAA and malic enzyme in the direction of malic acid decarboxylation. Therefore, increase in malic acid during ripening of banana is likely to be mediated by PEPC. But, there was no change in PEPC activity during ripening of banana as mentioned earlier. The results of the present investigation suggest a possibility of PEPC being under the control of sugar phosphates and organic acids in the fruit. Increase in the levels of activator G-6-P and the substrate PEP has been reported during the ripening of banana[18] and this might contribute to the accumulation of malic acid by enhancing PEPC activity. Thus the present work indicates a likelihood of PEPC being one of the regulatory enzymes in banana fruit metabolism. A similar regulation of PEPC activity has been demonstrated in other plants also.

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Dhal Recovery of Pulses Stored in Three Regions of Andhra Pradesh

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Dhal recovery of green gram, red gram, black gram and Bengal gram stored for one year in rural households of three regions of Andhra Pradesh (Telangana, Coastal and Rayalaseema) have been assessed. Periodical estimation of dhal yield has revealed that as the period of storage and level of insect infestation increased, the yield of dhal from the four pulses decreased and the yield of broken and husk increased. Decrease in dhal yield was more in red gram (7.79 per cent) followed by green gram (6.98 per cent) Bengal gram (3.04 per cent) and black gram (2.67) per cent) and among the regions Coastal region showed a higher percentage decrease in dhal yield except in green gram.

In Andhra Pradesh, though a small quantity of some pulses are consumed as whole gram, most of the pulses are consumed after processing into dhal[1]. Milling has been widely used as a means for decorticating pulses primarily those possessing a tough fibrous husk.

The dhal yield of pulses after milling in different parts of India varies as a result of varietal and agroclimatic characteristics. Bold grain varieties can be milled more easily than small and medium varieties and require less drastic premilling treatments[2]. Yield is also dependent on the cotyledon content of the varieties. Splitting and dehusking of grain is a varietal phenomenon influenced by moisture levels of the grain[3]. Not only varietal difference, the soundness of grain, extent of infestation will influence dhal yield. Hence the present study was undertaken to asses the dhal yield of stored pulses from three regions of Andhra Pradesh.

Materials and Methods

Selection of sample: Red gram (*Cajanus cajan*), green gram (*Phaseolus aureus*), Bengal gram (*Cicer arietinum*) and black gram (*Phaseolus mungo*) were selected for the study.

TABLE 1. REGIONWISE DISTRIBUTION OF SELECTED HOUSEHOLDS

Region	District	villages	No. of families
Telangana	Khammam	Tirumalaya palem	6
Coastal Andhra	Guntur	Eamani	6
Rayalaseema	Anantapur	Tadipathri	6

Area of study: The study was conducted in three regions of Andhra Pradesh namely, Telangana, Coastal and Rayalaseema regions. The regionwise distribution of selected households is given in Table 1.

Each pulse was selected from six households at random in each of the three regions. Pulses were collected (1 kg) from the farmers soon after harvest and after 4, 8 and 12 months of storage and yield of dhal, brokens and husk was assessed. Most commonly used methods for milling of pulses practiced among the families was selected and standardised in the laboratory. The milling of fresh and stored pulses was done in the laboratory following the standardised procedure. The details are given below.

Green gram (dry method): Oil (3.5 ml) was applied to the sample (1 kg) and the pulse was split in a stone *chakki* followed by drying continuously for two days. *Dehusking:* The split dhal was hand pounded and the husk was removed by winnowing.

Red gram (wet method): Gram was soaked in water for 5 hr and heaped in a corner, overnight. Afterwards it was dried continuously for three days and was split into dhal in a stone *chakki* and then dehusked; husk was removed by winnowing.

Black gram (dry method): Oil (3.5 ml) was applied to the sample (1 kg) and the pulse was split into dhal in a stone chakki. The split dhal was dried for two days.

Dehusking: The dried, split dhal was hand pounded and winnowed by a scoop.

Bengal gram (dry method): Sample was sun dried for two days and split into dhal in a stone chakki and then winnowed to remove the husk and brokens from the dhal.

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Before milling the sample, insect infestation, insect count and kernel damage were assessed by the method suggested by Pillai *et al*[4].

Results and Discussion

The dhal yield of the four pulses before and during storage differed significantly ($P \leq 0.05$). The yield of dhal, small brokens, big brokens and husk differed in samples collected from different regions in all the four pulses (Table 2, 3, 4 and 5). In green gram and red gram maximum dhal yield was obtained in samples from Telangana region while minimum dhal yield was obtained in samples from Rayalaseema region. In Bengal gram maximum dhal yield was observed in samples

TABLE 2. MILLING QUALITY OF FRESH AND STORED GREEN GRAM FROM THREE REGIONS OF ANDHRA PRADESH

Region	Dhal yield (g/kg) at diff. periods of storage			
	Initial	4 months	8 months	12 months
Telangana				
Dhal	677.33±10.81	650.00±20.00	643.00±23.30	626.67±22.11
Big brokens	47.50± 2.14	58.50± 3.40	61.33± 3.20	64.33± 3.73
Small brokens	65.17± 6.60	65.50± 3.55	66.00± 5.48	71.33± 3.40
Husk and powder	210.00±10.10	226.00±14.72	229.67±19.15	237.67±16.95
Coastal				
Dhal	674.50±19.43	645.83±19.23	636.17±25.82	622.67±11.06
Big brokens	52.16± 4.71	58.00± 3.81	63.67± 3.99	69.83± 3.76
Small brokens	60.67±10.14	68.00± 8.90	69.83± 6.77	73.50± 5.28
Husk and powder	212.67± 9.30	228.17± 6.09	230.33± 5.65	234.00± 6.81
Rayalaseema				
Dhal	641.50±11.98	629.50± 9.55	610.83± 7.13	603.33± 4.71
Big brokens	72.67± 4.71	73.67± 2.56	79.17± 3.67	79.33± 3.90
Small brokens	76.67± 2.43	79.67±13.49	82.67± 5.09	86.17± 3.97
Husk and powder	209.16±15.89	217.16±10.33	227.33± 7.50	231.17± 4.61

TABLE 3. MILLING QUALITY OF FRESH AND STORED RED GRAM FROM THREE REGIONS OF ANDHRA PRADESH

Region	Dhal yield (g/kg) at diff. periods of storage			
	Initial	4 months	8 months	12 months
Telangana				
Dhal	719.50±16.52	691.17±13.11	674.67±13.94	655.17±10.19
Big brokens	42.67± 2.75	48.00± 4.04	51.50± 5.47	56.67± 4.99
Small brokens	34.33± 5.09	42.00± 4.16	46.17±29.83	51.00± 2.45
Husk and powder	203.50±10.31	218.83±11.91	227.67± 8.08	237.17± 6.96
Coastal				
Dhal	690.67± 6.82	658.17± 9.82	637.83± 9.86	621.33± 5.02
Big brokens	46.33± 3.68	56.50± 3.50	61.67± 3.54	67.67± 3.14
Small brokens	37.67± 2.62	47.17± 6.18	50.83± 7.29	59.00± 5.26
Husk and powder	225.33± 4.31	238.16± 2.45	249.67± 4.27	252.00± 3.61
Rayalaseema				
Dhal	644.00± 5.92	632.83± 4.95	625.33± 8.71	615.67± 8.40
Big brokens	57.33± 2.69	59.17± 2.97	60.83± 5.96	63.00± 2.24
Small brokens	60.50± 2.14	63.67± 2.49	65.33± 3.59	64.50± 4.23
Husk and powder	238.17±11.82	244.33± 3.04	248.50± 5.25	256.83± 6.82

TABLE 4. MILLING QUALITY OF FRESH AND STORED BENGAL GRAM FROM THREE REGIONS OF ANDHRA PRADESH

Region	Dhal yield (g/kg) at diff. periods of storage			
	Initial	4 months	8 months	12 months
Telangana				
Dhal	675.17± 6.70	670.17±20.42	662.50±11.21	653.50± 8.60
Big brokens	42.50± 2.93	43.67± 3.50	44.00± 3.61	46.33± 1.94
Small brokens	42.17± 2.61	42.16± 3.85	43.17± 3.08	43.50± 3.20
Husk and powder	240.16± 4.98	244.00± 5.60	250.33± 7.54	256.67± 6.07
Coastal				
Dhal	693.17± 5.46	687.83± 8.28	679.00± 9.59	670.30±11.03
Big brokens	49.67± 5.29	49.50± 5.47	51.50± 6.02	53.67± 5.62
Small brokens	39.17± 6.42	38.00± 6.24	39.17± 4.84	41.67± 4.96
Husk and powder	218.00± 4.58	224.67± 5.09	230.33± 5.12	234.33± 7.36
Rayalaseema				
Dhal	680.83± 5.34	673.67± 5.35	668.17± 3.67	663.00± 5.16
Big brokens	50.67± 2.29	53.00± 2.38	54.00± 1.91	54.67± 1.49
Small brokens	34.33± 1.37	35.50± 1.38	36.83± 2.54	38.83± 3.44
Husk and powder	234.17± 3.53	237.83± 2.91	241.00± 2.65	243.50± 4.35

TABLE 5. MILLING QUALITY OF FRESH AND STORED BLACK GRAM FROM THREE REGIONS OF ANDHRA PRADESH

Region	Dhal yield (g/kg) at diff. periods of storage			
	Initial	4 months	8 months	12 months
Telangana				
Dhal	758.00±4.55	754.17±5.70	751.17±5.79	743.83±27.75
Big brokens	40.83±1.57	40.83±2.54	41.00±2.45	42.67± 2.21
Small brokens	40.33±3.22	41.17±1.68	41.33±1.60	42.83± 1.95
Husk and powder	160.83±3.80	163.83±4.45	166.50±3.35	170.67± 3.73
Coastal				
Dhal	727.67±5.22	720.83±7.82	710.00±9.61	703.17±10.35
Big brokens	35.33±2.21	37.67±3.45	40.50±4.75	42.83± 4.84
Small brokens	42.33±2.13	43.33±2.49	45.17±2.97	46.00± 4.20
Husk and powder	194.67±3.54	198.17±3.44	204.33±3.66	208.00± 4.04
Rayalaseema				
Dhal	772.67±7.09	766.83±9.37	758.00±5.13	751.17± 6.01
Big brokens	29.33±3.25	32.83±5.52	35.00±4.69	36.17± 4.52
Small brokens	31.00±4.40	29.00±7.09	31.50±7.11	32.83± 5.84
Husk and powder	167.00±2.38	171.33±6.34	175.50±4.79	179.83± 7.24

from coastal region followed by samples from Rayalaseema and Telangana. With regard to black gram maximum dhal yield was observed in samples from Rayalaseema and minimum in samples from coastal region.

It is also evident that as the period of storage and level of insect infestation increased, the yield of dhal decreased in all the four pulses (Table 6), with the consequent increase in the yield of brokens and husk (Table 2-5). Grains stored for a long time are generally considered easier to dehull (presumably due to drying) than the freshly harvested grains. When insect infestation is present in the grain as in the present study it will decrease the yield of dhal. The results are similar to those observed in literature[5,6]. As the larvae of bruchids develop

TABLE 6. THE EFFECT OF INSECT INFESTATION ON THE MILLING QUALITY OF FRESH AND STORED PULSES FROM THREE REGIONS OF ANDHRA PRADESH

Storage period (months)	Green gram			Red gram			Bengal gram			Black gram		
	Dhal yield (g/kg)	Insects/ 100 g	% kernel damage	Dhal yield (g/kg)	Insects/ 100 g	% kernel damage	Dhal yield (g/kg)	Insects/ 100 g	% kernel damage	Dhal yield (g/kg)	Insects/ 100 g	% kernel damage
Telangana Region												
0	677.3	—	—	719.5	—	—	675.2	—	—	758.0	—	—
4	650.0	10	4.0	691.2	5	2.2	670.2	5	1.6	754.3	5	1.4
8	643.0	19	7.6	674.7	16	5.0	662.5	7	2.5	751.2	9	1.9
12	626.7	36	14.8	655.2	30	13.1	653.5	17	31.	743.8	13	2.4
Coastal Region												
0	674.5	—	—	690.7	4	1.7	693.2	—	—	727.7	—	—
4	645.8	10	4.3	658.2	18	5.4	687.8	5	1.4	720.8	11	2.6
8	636.2	22	9.4	637.8	23	8.5	679.0	12	2.4	710.0	22	5.6
12	622.7	45	16.2	621.3	32	15.3	670.3	19	4.6	703.2	30	8.0
Rayalaseema Region												
0	641.5	—	—	644.0	—	—	680.0	—	—	772.7	2	0.4
4	629.5	—	3.1	632.8	7	2.7	673.7	5	0.8	766.8	7	1.4
8	610.8	20	8.0	625.3	14	4.5	668.2	11	2.1	758.0	21	5.1
12	603.3	37	15.5	615.7	31	8.8	663.0	17	3.7	751.2	26	6.0

inside the pulse and larval feeding is quite voracious. When they are milled, instead of pulses getting split into two halves, they become powder.

Among the four pulses, as the period of storage increased a higher decrease in dhal yield was observed in red gram followed by green gram, Bengal gram and black gram. Among the regions, samples from Costal region showed a higher percentage decrease in dhal yield except in green gram (Table 2-5). Samples from coastal region also showed a higher level of insect infestation when compared with the samples stored from the other two regions.

In the present study, the precentage increase in moisture content during storage in all the four pulses was more in the samples from coastal region (red gram 14.42 per cent, green gram 13.54 per cent, Bengal gram 23.32 per cent and balck gram 9.92 per cent). Grains absorb moisture above the safe level when the relative humidity of the surrounding air is high thus, becoming more susceptible to insect infestation. Though the grain was stored in ordinary gunny bags in all the regions, (which is not air tight) a higher moisture absorption was observed in samples stored in coastal region due to more humidity condition prevailing in that area.

In green gram, it was observed that at the end of twelve months storage period, the percentage decrease in dhal yield was lower (5.78 per cent) in the samples stored in Rayalaseema, where the level of insect infestation was found to be almost similar to that observed in coastal region.

Apart from insect infestation, there are several factors that arise out of varietal difference which also influence the milling characteristics of grains[3]. In green gram, two different types of local varieties were stored in Coastal and Rayalaseema regions namely green and black varieties which would definitely affect the dhal yield.

In conclusion, it can be stated that insect infestation does have an effect on dhal yield irrespective of the pulse stored.

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Low-lactose Infant Food From Buffalo Milk*

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An attempt was made to standardize a method for the manufacture of low-lactose infant food, either from coprecipitates (low, medium or high-calcium) or sodium caseinate obtained from buffalo skim milk, conforming to ISI specification of infant foods. A low-lactose infant food made from medium calcium coprecipitate, with unsalted buffalo milk butter and ground cane sugar was organoleptically acceptable. The infant food contained 24.2% protein, 24.7% fat, 4.5% ash and 40% carbohydrates including 2.1% lactose, a solubility index of 4.8 ml and a protein efficiency ratio of 2.7, as compared to 2.5 of casein.

Low-lactose milk and milk products can be prepared using different techniques like hydrolysis of lactose by enzyme lactase or β-galactosidase, fermentation of lactose by yeast and lactic cultures, lactose removal by gel filtration, ultrafiltration or reverse osmosis and use of lactose-free or low-lactose dairy ingredients like coprecipitates or caseinates. The last method offers greatest advantage by being relatively simple and less expensive as compared to others.

Unfortunately, there is no lactase preparations available in India. An attempt has been made to develop a low-lactose infant food (LLIF) from low, medium or high-calcium coprecipitates (LCC, MCC and HCC respectively) or sodium caseinate (SC) from buffalo milk while maintaining all the essential nutrients in proper proportion for optimum growth of infants.

Materials and Methods

Infant food manufacture: Low, medium and high-calcium coprecipitates were manufactured from buffalo milk by the method of Vijay Kumar and Gupta[2]. Sodium caseinate from buffalo milk, was made by the method of Webb and Whittier[3]. Low-lactose infant food was formulated by mixing coprecipitate or caseinate with melted, unsalted buffalo milk butter, heating (60 to 65°C) and homogenizing at 176 and 35 kg/cm² in a two stage homogenizer (Manton Gaulin, U.S.A.). The homogenized mix was spray dried maintaining inlet air temperature at 195±5°C and outlet air temperature at 95±5°C. Refined cane sugar ground to 30 mesh was dry mixed at the rate of 40 per cent on dry matter basis, with the spray dried mixture along with 0.6 g strawberry or pineapple or vanilla Capsoroma flavours (Naarden India Ltd., Bombay) per kg powder and packaged in 300 gauge (HDPE) polyethylene bags.

Analyses: The skim milk was analysed for total solids and fat content[4], titratable acidity and alcohol test[5], total nitrogen[6], and ash content[7]. The low-lactose infant food was analysed for moisture, ash, fat[8], protein[6], calcium[9], phosphorous[10], pH, solubility index[8], bulk density,[11] and lactose[7]. The reconstituted LLIF (14 g/100 ml water) was also analysed for its total solids[4] and pH.

The reconstituted LLIF was subjected to sensory

*Data taken from second author's M.Sc. Thesis, 1981, Kurukshetra University.

evaluation in terms of flavour, body and texture, colour and appearance using a 9-point hedonic scale score card.

The LLIF was also evaluated for protein efficiency ratio (PER) by the AOAC[12] method using male weanling albino rats fed *ad libitum* for 28 days on test and control casein[13] diet. The experimental diets consisted of 10 per cent protein, 8 per cent fat, 71 per cent starch, 5 per cent moisture, 5 per cent salt mixture and 1 per cent vitamin mixture[14], and contained 396 calories/100 g.

Results and Discussion

The skim milk used for the manufacture of coprecipitates and sodium caseinate contained 0.05 to 0.10 per cent fat, 9.65 to 10.12 per cent solids-not-fat, 3.45 to 3.85 per cent protein, 0.75 to 0.82 per cent ash and 0.14 to 0.15 per cent lactic acid. All the samples were negative for alcohol test.

The proximate composition of low-lactose infant foods made from coprecipitates or sodium caseinate is presented in Table 1, whereform it is evident that all formulations met the ISI-specifications[8] for infant food in terms of protein, fat, ash, carbohydrates and moisture contents. The solubility index was more than the maximum desirable, which could be attributed to the initial high solubility index of the buffalo milk coprecipitates[2]. The lactose content ranged from 1.8 to 2.9 per cent in the powder which, upon reconstitution with water (1:7) would yield a fluid containing 0.22 to 0.36 per cent lactose, being 15 to 20 times less than that present in milk. Although Buchanan and Henderson[15] were able to manufacture a carbohydrate free sterilized infant food from MCC or HCC, the lactose content in the infant food made by Skala and co-workers[16] was 0.07 per cent after reconstitution, which was slightly lower than those observed in this investigation. Vijay Kumar and Gupta[2] had observed that the lactose content of the various coprecipitates from buffalo milk was dependent upon the number of washings given to the coprecipitates curd. Hence it is possible to still further reduce the lactose content of these preparations by giving more than 3 washings to the curd.

TABLE 1. COMPOSITION AND CHARACTERISTICS OF LOW-LACTOSE INFANT FOODS (LLIF)

Characteristic	Low-lactose infant food based on				ISI specifications
	LCC	MCC	HCC	SC	
Moisture, (%)	4.3	4.3	4.5	4.4	Max. 4.5
Protein, (%)	26.8	24.2	24.6	24.2	Min. 20.0
Fat, (%)	22.3	24.7	24.2	24.8	18-28
Total ash, (%)	4.4	4.5	4.9	4.1	Max. 8.5
Carbohydrate, (%) (sucrose)	40.0	40.0	40.0	40.0	Min. 35.0
Lactose (%)	2.2	2.1	1.8	2.9	
Calcium, (mg %)	550.0	600.0	800.0	450.0	
Phosphorus,(mg%)	580.0	650.0	725.0	540.0	
Bulk density, (g/ml)	0.46	0.35	0.40	0.39	
Solubility index, (ml)	4.5	4.8	4.6	5.2	Max. 2.0
pH	7.1	7.0	7.1	7.1	

LCC=low calcium coprecipitate; MCC=medium calcium coprecipitate; HCC=high calcium coprecipitate; SC=sodium caseinate.

TABLE 2. OVERALL SENSORY SCORES OF RECONSTITUTED LOW-LACTOSE INFANT FOODS

Sample	Low-lactose infant food based on			
	LCC	MCC	HCC	SC
No added flavour (control)	6.4	6.6	6.7	6.5
Vanilla flavour	6.4	6.8	6.7	6.8
Strawberry flavour	6.8	6.9	6.6	6.7
Pineapple flavour	6.4	7.0	6.7	6.8

CD for sample: 0.232. Abbreviations as in Table 1.

The overall sensory score (Table 2) showed that in the absence of artificial flavouring, HCC based LLIF was liked best followed by that from MCC, SC and LCC. While with flavouring, the MCC based product was more acceptable than SC, HCC and LCC based ones. Buchanan and Henderson[15] also seemed to prefer MCC for their infant food formulation.

The nutritional evaluation (Table 3) showed that LCC based low-lactose infant food had the highest PER (3.3)

TABLE 3. PROTEIN EFFICIENCY RATIO OF LOW-LACTOSE INFANT FOODS

Type of infant food	Av. wt. gained (g)	Av. protein intake, (g)	Av. PER	Corrected PER
LCC	117.0	31.48	3.70	3.30
MCC	133.1	44.61	3.00	2.70
HCC	89.5	30.29	3.00	2.70
SC	122.6	40.16	3.00	2.70
Casein (control)	73.2	26.51	2.80	2.60

CD for diet: 0.256. Abbreviations as in Table 1.

as compared to ?.5 of casein, followed by 2.7 of the other three preparations, that is, MCC, HCC and SC based infant foods. Thus while the infant food samples had better PER than casein, the LCC based LLIF had significantly ($P<0.01$) higher PER than all other samples.

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On the ineral Content of Some Uncultivated Leguminous Seeds

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Ash samplained from seven leguminous seeds were analysed qualitatively and quantitatively for their mineral content. Pr of 19 elements (aluminium, barium, boron, calcium, cobalt, copper, iron, lead, magnesium, molybdenum, nickel, phos, potassium, silver, silicon, sodium, strontium, tin and zinc) have been detected and 16 elements quantitated. Pr of precious element silver in all seed ash has been reported for the first time. Silver, barium and lead occur t their bordering limit of spectroscopic estimation. *Sesbania grandiflora* seeds have been found richest in aluminiu nesium and phosphorus content.

In mammals n inorganic elements are known to be functional, one of them performing a different role in the bod study of the chemical composition of some uncult non-edible leguminous seeds revealed them as apply good sources of proteins and lipids along wne other nutrients[1-5]. However, no published d available on the mineral content of these seeds. efore, it was considered pertinent to make a co ive qualitative and quantitative study thereof in them by employing modern techniques.

Materials and Methods

Oven-dried seed powders (10 g) were ignited in a Therelek electric muffle furnance at 500°C to avoid loss of volatile elements with low melting points if any, present. For atomic absorption and flame emission analysis, the ash was evaporated to dryness twice over

with concentrated hydrochloric acid on a water bath, dissolved in hydrochloric acid (15 ml, 2N), filtered, washed the filter paper acid-free and the combined filtrate and washings were made up to 50 ml. This solution was employed for mineral analysis. The ash content of the seeds ranged from 3.0 to 4.7 per cent.

For qualitative and quantitative analysis by emission method, Hilger large glass and quartz spectrograph of British non-ferrous (BNF) source and Timer supplied by the same firm were employed. For atomic absorption, Hilger Unispek spectrophotometer with atomic absorption attachment was used. For alkali elements and calcium estimation Kipp and Zone flame photometer was employed. The spectra of elements were photographed on Ilford N40 photographic plates by giving 10 sec. exposure in each case.

The spectrum of each sample was obtained by employing the quartz spectrograph. The wave length of the various lines observed in the spectrum was determined and their identification made by reference to standard spectrum. Having thus identified the various lines for different elements, their quantitation in the test samples was made by measuring their photographic density and by comparing them with that (density) of the lines obtained from standard samples. Calibration curves were prepared by plotting the density of the lines under consideration with percentage concentration on logarithmic scale. The wave lengths of analysing lines in Å for aluminium, barium, lead, magnesium, molybdenum, nickel, silver, strontium and tin were, 3082.155, 5535.551, 2833.069, 2852.129, 3170.347, 3414.765, 3280.683, 4077.714 and 3262.328, respectively. Since the background was practically negligible, no correction was made for it.

The standards were prepared from specpure chemicals procured from M/s Johnson Matthey & Co. England. For the preparation and dilution of the standards a special flux developed in the laboratory of the Indian Bureau of Mines, Nagpur, consisting of SiO_2, Al_2O_3, Fe_2O_3, CaO, MgO, Na_2CO_3 and K_2SO_4 in the proportion of 63.0, 20.0, 6.0, 2.0, 2.0, 3.5 and 3.5 g/100 g mixture respectively was employed. The different constituents were mixed thoroughly and pulverized to a fine powder in an agate mortar and invariably used directly.

Aluminium, barium, lead, magnesium, molybdenum, nickel, silver, strontium and tin were assayed by arc emission method. Copper and zinc were estimated by atomic absorption spectro chemical method as described by Allan[6,7] and David[8,9]. Calcium and the alkali metals sodium and potassium were quantitated by flame photometric method as modified by Mitchell[10]. Phosphorus was estimated by Allen's[11] method and iron colorimetrically.

TABLE 1. QUALITATIVE SPECTROSCOPIC ANALYSIS OF SOME UNCULTIVATED LEGUMINOUS SEED FOR MINERAL CONTENT

Elements	Seed*						
	1	2	3	4	5	6	7
Aluminium	+	+	+	+	+	+	+
Barium	+	+	+	+	+	+	+
Boron	+	+	+	+	+	+	+
Calcium	+	+	+	+	+	+	+
Cobalt	—	Tr	—	—	—	+	Tr
Copper	+	+	+	+	+	+	+
Iron	+	+	+	+	+	+	+
Lead	+	+	+	—	—	+	—
Magnesium	+	+	+	+	+	+	+
Molybdenum	+	+	+	+	+	+	+
Nickel	+	+	+	+	+	+	+
Phosphorus	+	+	+	+	+	+	+
Potassium	+	+	+	+		+	+
Silver	+	+	+	+		+	+
Silicon	Tr	Tr	Tr	Tr		Tr	Tr
Sodium	+	+	+	+		+	+
Strontium	+	+	+	+		+	+
Tin	+	—	—	—		—	—
Zinc	+	+	+	+		+	+

Tr=Trace
+=Present

*1. *Bauhinia purpurea*
2. *Cassia glauca*
3. *Delonix regia* (*red*ered)
4. *Delonix regia* (*yel*lowered)
5. *Pongamia pinnata*
6. *Prosopis juliflora*
7. *Sesbania grandifl*

Silver, barium and lead bein[p]esent in their bordering limit of detection, the [q]titation thereof has been just mentioned.

Results and Discussion

The results of qualitative spectr[i]c analysis of all the seed ash samples have been [r]ded in Table 1 wherefrom it can be seen that the[s]les do not differ from one another with respec[t] their qualitative mineral components.

The presence of silver in all t[h] samples—a fact hitherto unreported in plants—[e]worthy. While cobalt occurs in *Cassia glauca*[,] *ppis juliflora* and *Sesbania grandiflora*, tin has be[e]cted only in the ash of *Bauhinia purpurea* whic[h] ther remarkable.

Table 2 records the quantita[t]a of the various elements in the seed ash sam[p]

TABLE 2. QUANTITATIVE ANALYSIS OF SOME UNCULTIVATED, LEGUMINOUS SEED ASH FOR MINERAL CONTENT

(Spectroscopic estimations expressed as p.p.m. and of chemical methods as g/100 g of seed ash)

Sl. No.	Elements	Seed* 1	2	3	4	5	6	7
1.	Aluminium	60	5	60	5	50	60	500
2.	Barium	10	10	10	10	10	10	10
3.	Lead	10	10	10	—	—	10	—
4.	Magnesium	1000	700	5000	600	5000	5000	5500
5.	Molybdenum	10	10	100	150	70	150	700
6.	Nickel	9	3	10	10	8	8	8
7.	Silver	1	1	1	1	1	1	1
8.	Strontium	10	60	30	50	10	50	60
9.	Tin	10	—	—	—	—	—	—
10.	Copper	3	2.75	9	3.5	3.0	2.5	7.0
11.	Zinc	3	3.60	5	4.0	3.5	4.0	3.5
12.	Calcium	10.5	200	22.7	13.0	11.5	10.0	10.5
13.	Potassium	22.0	170	22.7	20.0	26.0	21.5	25.0
14.	Sodium	2.8	10.7	5.5	1.5	1.4	5.0	3.3
15.	Iron	0.12	0.30	0.14	0.25	0.14	0.3	0.19
16.	Phosphorus	14.63	8.84	8.20	8.20	10.88	17.0	20.0

Sl. No. 1-9 by arc emission method
Sl. No. 10,11 by atomic absorption method
Sl. No. 12-14 by flame photometric method
Sl. No. 15,16 by chemical method

*1. *Bauhinia purpurea*
2. *Cassia glauca*
3. *Delonix regia* (red flowered)
4. *Delonix regia* (yellow flowered)
5. *Pongamia pinnata*
6. *Prosopis juliflora*
7. *Sesbania grandiflora*

Although all the seeds reveal the presence of aluminium, magnesium and molybdenum in appreciable quantities, *Sesbania grandiflora* concentrates them in maximum quantities. The highest percentage of copper and zinc has been detected in the seeds of *Delonix regia* (red flowered) whereas *Cassia glauca* exceeds all seeds in calcium, sodium and potassium content. While nickel and strontium occur in varying quantities in all seed samples barium, silver and lead occur only in just measurable quantities. *Cassia glauca* and *Prosopis juliflora* stand highest in iron content and phosphorus, although present in all seeds *Prosopis juliflora* and *Sesbania grandiflora* contain the maximum quantities thereof.

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Oxidimetric Determination of Dulcin (P-Ethoxy Phenyl Urea)

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Three new oxidimetric methods for the determination of dulcin (p-ethoxy phenyl urea), a non-nutritive sweetener, have been developed using the oxidants cerium (IV), vanadium (V) and hexacyano ferrate (III). The above reactions are stoichiometric consuming 26, 10 and 10 moles of the oxidant respectively per mole of dulcin under the experimental conditions. The recovery of added dulcin to synthetic ready-to-serve beverages has been reported.

Dulcin (p-ethoxy phenyl urea), a non-nutritive sweetener, prohibited under the provisions of Prevention of Food Adulteration Act, 1954 and Rules 1955 thereof[1] is still being used in many food products such as beverages, ice cream, ice candy, etc, Methods proposed earlier for its determination include colourimetric[2-6], UV-spectrophotometric[7] and fluorimetric[8,9] but so far no volumetric method has been developed. Simple and convenient oxidimetric methods are developed for the first time using the oxidants such as ceric sulphate, sodium vanadate and hexacyanoferrate for its determination. Back titration procedures are adopted as the reaction is not rapid enough for direct titrations at room temperature.

Materials and Methods

All the reagents used were of A.R./G.R. grade. Decinormal reagents of ceric sulphate, sodium vanadate (from ammonium meta vanadate), potassium hexacyano ferrate, ferrous ammonium sulphate, sodium thiosulphate and dulcin (1mg/ml) were prepared in double distilled water. 0.1 per cent N-phenyl anthranillic acid (NPA) was prepared by dissolving it in minimum amount of sulphuric acid.

Procedure using ceric sulphate: An aliquot of dulcin solution was mixed with 20ml of ceric sulphate solution in a 250 ml conical flask fitted with a standard joint and requisite volume of sulphuric acid was added to maintain the overall acidity of 6N when diluted to 50 ml. The mixture was refluxed on a hot plate for 150 min. After cooling, it was titrated against standard ferrous ammonium sulphate using NPA as indicator.

Procedure using sodium vanadate: An aliquot of dulcin solution was mixed with 20 ml of sodium vanadate and the overall acidity was maintained at 4 to 6N using hydrochloric acid in a volume of 50 ml. The reaction mixture was kept at room temperature (28-29°C) for 120 min. It was then titrated against standard ferrous ammonium sulphate using NPA as indicator.

Procedure using hexacyano ferrate (III): An aliquot of dulcin solution was mixed with 20 ml of hexacyano ferrate and the overall alkalinity was maintained at 0.2N using 2 N sodium hydroxide when diluted to 50 ml. The reaction mixture was kept in a boiling water bath for 60 min. After cooling, the contents of the flask were acidified with hydrochloric acid, 10 ml of 20 per cent potassium iodide and 2 g. of zinc sulphate were added and the liberated iodine was titrated against standard sodium thiosulphate using starch as indicator.

The amount of dulcin (X mg) present is given by $X = \frac{M}{n} N (V_1 - V_2)$ where, M is the molecular weight of dulcin (180.20), N is the normality of the titrant used, V_1 is the blank titer and V_2 is the titer for the sample. n is the number of electrons involved in the reaction (26, 10 and 10 respectively for cerium (IV), vanadium (V) and hexacyano ferrate (III).

Procedure for the determination of dulcin in beverages: An appropriate quantity of the beverage containing 5-15 mg of dulcin was taken into a separatory funnel, made alkaline using 5 ml of 10 per cent sodium hydroxide and extracted with 3×40 ml portions of diethyl ether. The combined ether extract was washed free of alkali and taken into a conical flask fitted with a standard joint. The solvent was removed and the above mentioned procedures were followed for estimation.

Results and Discussion

The working ranges of dulcin under the set experimental conditions are found to be 5-25 mg in case of

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TABLE 1. RECOVERY OF DULCIN BY THE PROPOSED OXIDIMETRIC METHODS

Dulcin added (mg)	mg*		
	Cerium (IV) Mean±S.D.	Vanadium (V) Mean±S.D.	Hexacyano ferrate (III) Mean±S.D.
5.0	5.02±0.03	5.03±0.04	5.04±0.06
10.0	10.03±0.05	10.00±0.03	9.98±0.02
15.0	14.96±0.12	14.89±0.32	14.64±0.64
18.0	17.92±0.51	17.84±1.20	16.26±1.86
20.0	19.64±0.62	19.50±1.62	—
25.0	24.74±0.92	—	—

*Values are the mean of six analyses

cerium (IV), 5-20 mg in case of vanadium (V) and 5-15 mg in case of hexacyano ferrate (III). The recovery of dulcin taken in different ranges, by the proposed oxidimetric procedures is given in Table 1. The percentage recovery ranged from 98.2 to 100.4, 97.5 to 100.6 and 97.6 to 100.8 in case of oxidants cerium, vanadium and hexacyano ferrate respectively, which gives a good scope for its purity assay. The recoveries of added dulcin to synthetic ready-to-serve orange flavoured and lime flavoured beverages in concentration ranging from 100 to 500 ppm given Table 2, are found satisfactory.

The high oxidation potentials and the stability at higher temperatures of these oxidants are the reasons for their selection. In case of oxidation using cerium (IV), the consumption of 26 moles of the oxidant per mole of dulcin may be explained taking into consideration that under drastic conditions dulcin undergoes hydrolysis leading to the formation of p-amino phenol and ethanol[10], of which p-amino phenol oxidation consumes 22 moles[11] and 4 moles for ethanol oxidation. This is further confirmed by adding a known quantity of ethanol to the oxidation mixture and establishing excess four moles. The consumption of 10 moles each of sodium vanadate and hexacyano ferrate per mole of dulcin may be explained taking into consideration of the requirement of 6 moles for p-amino phenol (oxidative coupling reaction and degradation)[12] and four moles for ethanol oxidation.

TABLE 2. PERCENT RECOVERY* OF DULCIN ADDED TO SYNTHETIC (ORANGE AND LIME FLAVOURED) RTS BEVERAGES

Name of the product	Dulcin added (ppm)	Cerium (IV) Mean±S.D.	Vanadium (V) Mean±S.D.	Hexacyano ferrate (III) Mean±S.D
Water (control)	100	100.6±0.6	101.0±0.9	100.8±0.7
-do-	500	99.0±0.2	98.6±0.4	98.5±0.6
Orange beverage	100	101.0±0.8	101.3±1.1	101.8±1.7
-do-	200	97.9±1.0	97.6±1.2	97.0±1.9
Lime beverage	100	100.8±0.9	101.0±1.3	100.6±0.7
-do-	500	98.4±0.7	97.4±1.6	97.0±1.9

*Values are mean of five analyses

Carbohydrates, which readily undergo oxidation with all these oxidants should be absent. Salicylic acid and caffeine are found to interfere in these estimations resulting in a positive error. The interference due to salicylic acid and other phenolic compounds can easily be avoided as these compounds will not be extracted under alkaline conditions into the solvent. Anions like chloride, sulphate and phosphate did not interfere even when they were present in 10 fold molar excess to that of dulcin.

The methods suggested are accurate, simple, makes use of commonly available chemicals and can be used for purity assay and estimation in beverages.

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Activated Clay as a Seed Protectant. I. Studies on the Damage by *Ephestia cautella* Walker and *Trogoderma granarium* Everts and Its Effect on the Germination of Wheat Seeds Treated with Clay

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The effect of different types of clay on the germination of seed and the damage of *Ephestia cautella Walker* and *Trogoderma granarium* Everts and effect of damage on germination of wheat seeds was studied. The following conclusions were drawn: (1) Germination was not impaired in seeds treated with clay. Moreover, 0.1 per cent level of AC III (activated H. kaolinite), AC IV (activated conditioned kaolin) and AC VII (partially activated Ca kaolin-talc formulations) indicated good effect on germination. (2) The damage caused by *T. granarium* affected the germination of seed treated with AC V (activated Ca kaolin) and AC VI (partially activated kaolin-talc formulations) at levels of 0.3, 0.5, 1.0 and 1.5 per cent and seeds treated with AC VII at 0.3 per cent level, 25 days after releasing larvae in treated seeds. Whereas, normal germination was significantly reduced in seed treated with AC III and AC IV at 0.3, 0.5 and 1.0 per cent levels, 30 days after releasing larvae. (3) AC III and AC IV at 1.5 per cent could be used to protect seeds of wheat against the infestation of *T. granarium*. (4) In seed treated with AC VII and AC V at 1.5 per cent level normal germination was not affected due to damage caused by *E. cautella*. This was also observed in seed treated with AC III and AC IV at 1.0 per cent level. Significantly poor germination was recorded in seed treated with AC V at 0.3, 0.5 and 1.0 per cent levels. In seed treated with AC VI at all levels, normal germination was significantly affected due to damage caused by the developing larvae of *E. cautella*.

Deterioration in storage by stored grain pests is one of the important problems in maintaining quality of seeds of wheat in common with other cereals which are often infested by *E. cautella* and *T. granarium* in stores[1]. The critical level of infestation after which loss in germination occurs in the seed is not known. Limited data are available on the damage caused by pests and its effect on the germination of wheat seed[2-5]. However, susceptibility of different varieties and strains of wheat to insect infestation has been reported by Chatterjee[6], Bhatia and Gupta[7] and Singh *et al.*[8,9] No attention has been paid so far to elucidate the effect of mineral compounds on insect and its effect on seed germination. A test has been made, therefore, with *E. cautella* and *T. granarium* and to determine their infestation and its effect on the germination of wheat treated with different types of clay.

Materials and Methods

Seeds of wheat were separately treated to give 0.3, 0.5, 1.0 and 1.5 per cent levels of each type of clay, AC III (activated H. kaolinite), AC IV (activated conditioned kaolin), AC V (activated Ca kaolin), AC VI (partially activated kaolin-talc formulations) and AC VII (partially activated Ca kaolin-talc formulations) and then stored in glass jars in the laboratory at 26.5-30°C and 60-65 per cent relative humidity.

The method of screening the seeds was the same as described by Singh *et al.*[5] One hundred fifty grams of sound treated seeds were kept in glass jars (11b). Fifty, 24-hr old larvae of each insect were released to each glass jar containing the seeds. The jars were covered with muslin cloth and kept in desiccator at 75 per cent R.H.[10] Each concentration of clay was replicated four times and an untreated check served as control. 15, 25, 30 and 40 days after releasing the larvae, the seeds were subjected to high temperature (55°C) in an oven for 4 hr to check further development of larvae[4]. The untreated seeds were also similarly heated. One set of each treatment was kept to observe the percentage of seed infested by the larvae of *T. granarium* and *E. cautella*. After 40 days, the percentage of infested seeds in each replication was recorded.

After heat treatment, germination tests were carried out[11]. Germination was categorised into two groups, viz., (*i*) normal germination where epicotyl and hypocotyl emerged normally and showed satisfactory growth

upto two weeks and (*ii*) abnormal germination where epicotyl and hypocotyl were damaged or where no germination took place. As the germination in control was not hundred per cent, the percentage germination in infested seeds was also calculated accordingly. The formula reported by Singh *et al.*,[5] was used to find out the reduced percentage of germination. The percentage germination in uninfested seeds were statistically, analysed, as calculated by Campbell[12]. The germination of treated seeds after 2, 4 and 6 months of storage was also studied.

Results and Discussion

The results show that AC IV at 1.0 and 1.5 per cent levels was significantly superior to other treatments. The damaged seeds in the above were 0.00-1.5 and 2.0-3.5 per cent as against 1.5-15.0 and 2.5-20.2 per cent in untreated seeds caused by *E. cautella* and *T. granarium* respectively (Table 1). Seeds treated with AC III at 1.0 and 1.5 per cent levels showed significantly poor infestation by *T. granarium*. *T. granarium* did not show partially infested seeds at 1.0 per cent level of AC III. The percentage of partially infested seeds increased in AC III with 0.3 to 0.5 and 1.0 to 1.5 per cent levels. Seeds with germ infestation caused by *E. cautella* decreased in those treated with AC III. AC IV and AC VII at 0.3 to 1.5 per cent levels. The partially infested seeds caused by *T. granarium* was recorded only in seeds treated with AC III at 1.5 per cent level. Significantly high infestation by both the species was observed in seeds with AC V and AC VI at all the levels tested.

The results on the viability of seeds treated with different types of clay are given in Table 2. The results

TABLE 1. PERCENTAGE INFESTATION CAUSED BY DEVELOPING LARVAE OF *E. CAUTELLA* AND *T. GRANARIUM* IN DIFFERENT TYPES OF CLAY TREATED WHEAT GRAINS

Type of clay*	Dosage (%)	Percentage of damaged kernel			
		E. cautella		*T. granarium*	
		Germ eaten	Partially eaten	Germ eaten	Partially eaten
AC III	0.3	5.50	2.00	6.50	0.00
	0.5	5.60	2.50	6.50	0.00
	1.0	2.50	2.50	5.00	0.00
	1.5	1.50	3.50	2.50	1.50
	Mean	3.65	2.65	5.13	0.38
AC IV	0.3	3.50	1.00	5.20	0.00
	0.5	2.00	2.50	5.00	0.00
	1.0	0.00	1.50	3.50	0.00
	1.5	3.00	0.00	2.00	0.00
	Mean	1.38	1.25	5.93	0.00
AC V	0.3	8.50	2.50	12.00	0.00
	0.5	8.20	2.50	12.50	0.00
	1.0	7.50	1.00	11.50	0.00
	1.5	7.00	0.00	11.50	0.00
	Mean	7.80	1.50	11.88	0.00
AC VI	0.3	0.00	1.50	11.00	0.00
	0.5	8.50	2.00	10.50	0.00
	1.0	8.50	1.00	10.50	0.00
	1.5	7.50	1.50	9.50	0.00
	Mean	8.38	1.50	10.38	0.00
AC VII	0.3	5.50	2.50	5.50	0.00
	0.5	3.50	7.50	5.80	0.00
	1.0	3.50	3.00	5.00	0.00
	1.5	1.50	3.50	2.50	0.00
	Mean	3.50	2.88	4.55	0.00
Control		16.00	1.50	20.20	2.50

*For details of clay types see text.

TABLE 2. VIABILITY OF WHEAT SEEDS (PER CENT) TREATED WITH DIFFERENT LEVELS OF CLAYS

Type of clay	Dosage (%)	Period of storage in months		
		2	4	6
AC III	0.3	76.31	84.37	84.35
	0.5	83.33	93.93	95.50
	1.0	90.56	96.55	98.25
	1.5	80.09	95.53	95.50
	Mean	82.57	92.60	95.40
AC IV	0.3	78.78	87.75	87.00
	0.5	88.88	89.74	89.75
	1.0	91.66	100.00	99.00
	1.5	84.84	89.74	90.00
	Mean	86.04	91.80	91.43
AC V	0.3	56.52	85.71	85.00
	0.5	66.66	81.48	81.50
	1.00	69.69	90.24	90.55
	1.5	83.72	94.11	95.00
	Mean	69.14	87.88	88.01
AC VI	0.3	80.85	80.75	80.70
	0.5	85.36	86.54	87.00
	1.0	89.74	88.74	88.50
	1.5	91.66	92.00	95.00
	Mean	86.90	87.00	87.80
AC VII	0.3	83.57	98.00	98.50
	0.5	88.88	98.00	96.50
	1.0	95.09	100.00	90.55
	1.5	87.75	98.00	98.50
	Mean	88.82	98.50	96.01
Control	—	76.47	79.05	79.00

Each value is the mean of four.

show that viability was not impaired in any of the treatments after 2, 4 and 6 months of treatment. AC III, AC IV and AC VII showed no inhibitory effect on germination at 0.3-1.0 per cent levels, whereas 1.5 per cent level produced inhibitory effect on germination. This trend of inhibition of germination of seeds was not noticed in those treated with AC V and AC VI.

The relationship between the extent of damage caused by the developing larvae of *E. cautella* and *T. granarium* and percentage reduction in germination of seeds treated

TABLE 3. PERCENTAGE REDUCTION IN GERMINATION OF WHEAT SEEDS DUE TO DAMAGE CAUSED BY DEVELOPING LARVAE OF *E. CAUTELLA* TREATED WITH DIFFERENT LEVELS OF CLAY

Type of clay	Dosage (%)	Damage (%)	% germination in uninfested seed	Confidence Interval Limit	% germination after indicated days of larvae release			
					15	25	30	40
AC III	0.3	7.5	94.3	81.0-86.8	84.5 (0.0)	84.3 (0.0)	83.0 (20.0)	82.5 (27.0)
	0.5	7.5	92.0	89-5-94.5	92.0 (0.00)	92.0 (0.00)	91.5 (7.60)	91.0 (15.30)
	1.0	5.0	95.5	93.0-97.0	95.3 (.00)	95.5 (.00)	95.5 (0.00)	95.3 (4.00)
	1.5	5.0	93.5	91.0-96.0	93.5 (0.00)	93.3 (0.00)	93.5 (0.00)	93.5 (0.00)
AC IV	0.3	4.5	80.0	87.5-82.5	80.0 (0.00)	80.0 (0.00)	79.7 (6.66)	79.5 (11.11)
	0.5	4.5	85.0	83.0-88.0	85.5 (0.00)	85.5 (0.00)	85.3 (4.44)	85.10 (11.11)
	1.0	2.5	92.0	89.5-94.5	92.0 (0.00)	92.0 (0.00)	92.0 (0.00)	91.9 (4.00)
	1.5	0.00	88.0	85.5-90.5	88.0 (0.00)	88.0 (0.00)	88.0 (0.00)	88.0 (0.00)
AC V	0.3	11.0	85.7	85.7-88.2	85.0 (6.31)	84.5 (10.9)	83.5 (20.0)	81.0 (42.72)
	0.5	10.7	83.5	81.0-86.0	83.2 (2.8)	83.0 (4.67)	82.7 (7.47)	81.0 (20.56)
	1.0	8.5	90.0	87.5-92.5	90.0 (0.00)	89.6 (4.70)	89.5 (5.80)	89.0 (11.75)
	1.5	7.0	93.2	90.7-95.7	93.2 (0.00)	93.2 (0.00)	93.0 (2.85)	93.0 (2.85)
AC VI	0.3	10.5	87.7	92.6-97.5	87.0 (6.66)	37.0 (6.66)	85.5 (25.71)	83.2 (40.00)
	0.5	10.5	88.7	86.25-91.25	88.5 (1.90)	38.5 (6.66)	87.7 (9.52)	86.5 (23.80)
	1.0	9.5	88.7	86.25-91.25	88.6 (1.05)	38.5 (2.10)	87.8 (9.47)	87.0 (17.80)
	1.5	9.5	95.5	93.0-98.0	95.5 (0.00)	95.0 (3.26)	95.0 (3.26)	94.8 (7.36)
AC VII	0.3	8.0	88.0	85.5-90.5	88.0 (0.00)	88.0 (0.00)	87.5 (0.25)	86.8 (15.00)
	0.5	6.0	91.2	88.7-93.7	91.2 (0.00)	91.2 (0.00)	91.0 (5.16)	90.5 (12.50)
	1.0	6.5	98.0	95.5-100.5	98.0 (0.00)	98.0 (0.00)	97.8 (3.07)	97.5 (7.60)
	1.5	6.5	95.5	93.0-98.0	95.5 (0.00)	95.5 (0.00)	95.5 (0.00)	95.3 (3.07)
Control		16.5	94.0	81.5-86.5	92.5 (9.09)	81.0 (18.18)	79.5 (27.27)	75.2 (53.33)

The figures in parantheses denote the average percentage reduction in germination due to insect infestation.

TABLE 4. PERCENTAGE REDUCTION IN GERMINATION DUE TO DAMAGE CAUSED BY *T. GRANARIUM* OF WHEAT SEEDS TREATED WITH DIFFERENT LEVELS OF CLAY

Type of clay	Dosage (%)	Damage (%)	% germination in uninfested seed	Confidence Limit	% germination after indicated days of larvae release			
					15	25	30	40
AC III	0.3	6.5	84.3	81.8-86.8	84.0 (4.60)	84.0 (4.60)	83.5 (12.30)	83.0 (20.00)
	0.5	6.5	92.0	89.5-94.5	92.0 (0.00)	91.7 (4.60)	91.5 (7.70)	91.5 (7.79)
	1.0	5.0	95.5	93.0-98.0	95.5 (0.00)	95.5 (0.00)	95.2 (6.00)	95.1 (8.00)
	1.5	4.0	95.0	92.5-97.5	95.0 (0.00)	95.0 (0.00)	94.9 (2.50)	94.7 (7.50)
AC IV	0.3	5.2	80.0	77.5-82.5	80.0 (0.00)	79.8 (3.80)	79.5 (9.60)	79.0 (19.23)
	0.5	5.0	85.5	83.0-88.0	85.5 (0.00)	85.3 (4.00)	85.2 (6.00)	85.0 (10.00)
	1.0	3.5	92.0	89.5-94.5	92.0 (0.00)	92.0 (0.00)	91.8 (5.70)	91.7 (8.60)
	1.5	2.0	88.0	85.5-90.5	88.0 (0.00)	88.0 (0.00)	88.0 (0.00)	87.9 (3.00)
AC V	0.3	12.0	85.7	83.2-88.7	85.0 (5.80)	84.5 (10.00)	84.2 (12.50)	83.0 (22.50)
	0.5	12.5	83.5	81.0-85.0	83.2 (2.40)	82.8 (5.60)	82.0 (12.00)	82.0 (12.00)
	1.0	11.5	90.0	87.5-92.5	89.8 (1.70)	89.5 (4.50)	89.0 (8.70)	88.8 (10.4)
	1.5	11.5	93.2	90.7-95.5	93.2 (0.00)	93.0 (1.70)	82.7 (4.30)	82.5 (6.10)
AC VI	0.3	11.5	87.7	85.2-90.2	87.0 (6.18)	86.8 (7.80)	86.0 (14.8)	85.5 (19.10)
	0.5	11.5	88.7	86.2-91.2	88.4 (2.60)	88.0 (5.10)	87.5 (10.4)	87.0 (14.80)
	1.0	10.5	88.7	86.2-91.2	88.5 (1.90)	88.0 (6.70)	87.5 (11.40)	87.2 (14.30)
	1.5	9.5	95.5	93.0-98.0	95.3 (2.10)	95.0 (5.60)	94.8 (7.40)	94.0 (15.80)
AC VII	0.3	5.5	88.0	85.5-90.5	87.8 (3.60)	87.6 (7.30)	87.5 (9.10)	87.0 (18.20)
	0.5	5.2	91.2	87.7-91.0	91.0 (3.80)	91.0 (3.80)	90.8 (7.70)	90.8 (7.70)
	1.0	5.0	98.0	95.5-100.5	98.2 (0.00)	98.0 (4.00)	97.8 (8.00)	97.8 (8.00)
	1.5	2.5	95.5	93.0-98.0	95.5 (0.00)	95.5 (0.00)	95.4 (4.00)	95.2 (8.00)
Control		16.5	84.0	81.5-86.5	82.5 (9.0)	81.0 (18.18)	79.5 (27.20)	75.2 (53.53)

The figures in parentheses denote the average percentage reduction in germination due to insect infestation.

with different types of clay at 0.3, 0.5, 1.0 and 1.5 per cent levels are presented in Tables 3 and 4.

The data reveal that in seeds treated with AC III and AC VII at 0.3 and 0.5 per cent levels normal germination was significantly affected. AC IV at 0.3 per cent level significantly affected the germination only after 30 days

If releasing the larvae. Whereas in those treated with 0.5 per cent level of AC IV, normal germination was significantly affected 40 days after releasing the larvae. In seeds treated with AC III and AC IV at 1.0 and 1.5 per cent level, normal germination was not affected until the completion of development of larvae of *E. cautella*. The data further show that in seeds treated with AC V and AC VI (0.3 per cent level) the normal germination was significantly affected 15 days after releasing the larvae and by the completion of development of the larvae a corresponding reduction of 42.72 and 40.00 per cent germination was observed. In those treated with AC VI (0.5 per cent level), the normal germination was significantly affected only 30 days after releasing the larvae, whereas in seeds treated with AC V (1.5 per cent level) the normal germination was not significantly affected even 40 days after releasing the larvae.

The percentage germination due to damage caused by the developing larvae of *T. granarium* (Table 4) was significantly affected in seeds treated with AC III and AC IV at 0.3, 0.5 and 1.0 per cent level 30 days after releasing the larvae. In seeds treated with AC V and AC VI, the normal germination was significantly affected 15 days after releasing larvae and in seeds treated at 0.5, 1.0 and 1.5 per cent level of AC VI, the normal germination was significantly affected 25 days after releasing the larvae. In case of seeds treated with AC III and AC VII (1.5 per cent level) the normal germination was significantly reduced only 40 days after releasing the larvae. No significant reduction in normal germination was observed in seeds treated with AC IV at 1.5 per cent level due to damage caused by *T. granarium*.

It is concluded that treatment with AC IV at 1.0 and 1.5 per cent levels resisted the infestation by *E. cautella*. The seeds were also resistant to infestation by *T. granarium*. Significantly high infestation was recorded by both species of pests in seeds treated with AC V and AC VI. AC III, AC V and AC VII at 1.0 per cent level did not impair the germination of seeds. No significant reduction in normal germination of seeds by *E. cautella* was observed in seeds treated with AC III, and AC IV at 1.0 and 1.5 per cent levels. Moreover, AC V and AC VII showed protective action to the damage caused by *E. cautella*. Whereas none of the treatment of AC VI could provide protection to the seeds of wheat against the damage caused by *T. granarium* and *E. cautella*.

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Influence of Capsaicin on the Absorption of Amino Acids and Fat in Rats

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The absorption of groundnut oil in rats was not affected by capsaicin (1.5 or 4.5 mg/rat) either when it was administered by gavage along with the oil or when it was fed at 15 mg% in the diet for 6 weeks. Similarly, the absorption of three representative amino acids viz., alanine, histidine and glutamic acid was also not adversely influenced by capsaicin as indicated by the determination of left over amino acids in the G-I tract of rats, maintained for 4 weeks on a 15 mg% capsaicin diet or in the mucosal medium in which everted intestinal sacs prepared from rats on capsaicin diet were incubated. Further when capsaicin (7.5 or 15.0 mg%) was added to the medium in which everted intestinal sacs from rats on a control diet were incubated, there was no adverse influence on amino acid absorption.

Red pepper (*Capsicum annum* L.) is a common spice consumed by people all over India. The whole spice, its active principle 'Capsaicin' (8-methyl N-vanillyl 6-nonenamide) and the synthetic analogue (N-vanillyl nonanamide) have been investigated in this laboratory[1] for their influence on growth, food efficiency ratio and blood constituents. There were no adverse effects on growth, food intake, weight gain or blood constitutents noticeable in short or long duration feeding. Both were found to have no influence on nitrogen absorption. Nitrogen retention was significantly high in 0.5-2.0 per cent red pepper fed groups. It was significantly lower in 5 per cent red pepper, and 3 and 15 mg per cent capsaicin fed groups. As part of our general plan on the influence of various spices and their active principles on the absorption of major nutrients and with a view to seek explanation for the lowered nitrogen retention, the influence of capsaicin on amino acid absoprion was studied. An earlier report[2] on the retardation of growth in rats by capsaicin due to its lowering of fat absorption, also prompted us to study this aspect as well.

The results of experiments on the absorption of amino acids and fat in (*i*) rats fed by gavage along with or without capsaicin, and in (*ii*) those maintained on control, and control+capsaicin diets are reported in this communication.

Materials and Methods

Synthetic capsaicin (N-vanillyl-5-nonanamide) was obtained from FLUKA-AG, Switzerland. DL-alanine U-^{14}C (sp.act. 33 mCi/m mole) and L-glutamic acid U-^{14}C (sp. act. 107.5 mCi/m mole) were obtained from BARC, Bombay, India. L-Histidine 2^{14}C (sp. act. 50 mCi/m mole) was from Radio Chemicals Centre, Amersham, England. All other chemicals and materials of required standard were obtained from local sources.

Groups of adult male Wistar rats (150-200 g body weight) were fed (1) the basal[3] diet serving as control group in all the experiments and another (2) basal+15 mg per cent capsaicin for 4-6 weeks.

Amino acid absorption: (*i*) *In vivo:* A saline solution containing 100 mg each of the labelled amino acids was administered by gavage to two groups of fasted (overnight) animals. To one set of animals capasaicin (1.5 mg per rat) dissolved in groundnut oil (1.0 ml) and to the other plain oil was administered along with the the amino acid. One hour later, the animals were killed by decapitation, the abdomen was cut open and ligatures were made at the ileo-caecal junction and at the stomach-oesophagus junction. The entire gastro-intestinal tract was removed, thoroughly flushed with saline and washings made upto a definite volume.

(*ii*) *In vitro:* After decapitation, portions of small intestine representing the jejunal region were excised from animals fed for 4 weeks either with control or the control+15 mg per cent capsaicin containing diets. The lumen contents were gently flushed with isotonic saline and the intestine quickly everted on a thin glass rod. The everted sacs (5 cm) were filled with Krebs-Ringer phosphate buffer of pH 7.4 containing 0.2 per cent glucose. In experiments where capsaicin was added to the incubation medium, adjacent sacs were used as

control. Capsaicin was added as suspension in phosphate buffer-Tween 20. Incubations were carried out in 50 ml Erlenmeyer flasks containing 10 ml of the above buffer to which the amino acid had been added at 10mM concentration. Flasks were aerated with O_2-CO_2 (95, 5 V/V) for 10 min before incubation in a Dubnoff-Metabolic shaking incubator for 30 min at 37°C at about 60 cycles/min. After incubation, the sacs were removed, the fluid adhering to the mucosal surface was washed into the medium and the serosal fluid and tissue were collected separately.

Determination of radioactivity: Aliquots of the mucosal and serosal fluids were counted using Bray's solution in a Packard Tricarb-Prias counter. The tissue was solubilized in 1.5 ml of Soluene-350 and aliquots were counted similarly.

Fat absorption: For determining fat absorption, groundnut oil without or with capsaicin (1.5 or 4.5 mg/rat) was administered by gavage. In the experiment where the influence was studied in animals maintained for 6 weeks on diet containing capsaicin (15 mg per cent) or the control diet, fat was administered similarly. After a lapse of 2 hr all animals were killed by decapitation, intestinal and stomach contents were washed and the washings made upto volume and analysed as described by Irwin *et al.*[4]

Statistical analysis: Differences in absorption caused by capsaicin were evaluated using students 't' test[5].

Results and Discussion

The influence of capsaicin on the absorption of three representative amino acids, alanine, histidine and glutamic acid is shown in Table 1. Absorption of the individual amino acid is not affected in any of the cases (*i*) from gastrointestinal tract or everted sacs of control rats in the presence of capsaicin or from the G.I. tract of rats maintained on capsaicin containing diets for 4 weeks. The uptake by everted sacs from rats on the conrol diet is similar to what has been reported by Finch and Hird[6]. The absorption of glucose in rats is reported to be stimulated at low (7-10 mg per cent) or inhibited at high (14-21 mg per cent) concentration of capsaicin[7]. Capsaicin added at two different concentrations to the mucosal media in which everted sacs of control rats were incubated also did not affect amino acid absorption.

Studies on fat absorption were carried out *in vivo*

TABLE 1. INFLUENCE OF CAPSAICIN ON ABSORPTION OF AMINO ACIDS IN RATS

	In vivo Absorption from G. I. tract		*In vitro*[a] Uptake by everted sacs		*In vitro*[b] Uptake by everted sacs	
	m moles/hr	%	μM/g dry tissue/30 min	%	μM/g dry tissue/30 min	%
Alanine						
Control	0.87±0.03	77.96	112.68±17.51	7.90	33.25±5.50	4.77
Test	0.76±0.06	67.78	130.40± 8.78	7.59	33.11±1.44*	4.53*
					31.94±1.44**	4.40**
Histidine						
Control	0.55±0.01	94.40	82.37± 5.22	5.70	39.66±3.61	3.36
Test	0.54±0.01	93.20	100.80±12.67	5.94	35.90±0.01*	3.23*
					35.89±2.84**	2.93**
Glutamic acid						
Control	0.54±0.02	79.20	149.80± 8.70	10.20	29.86±2.72	4.01
Test	0.56±0.03	82.70	165.65±11.32	9.97	32.90±1.99*	4.51*
					40.32±2.12**	5.56**

Values are mean±S.E.M. for 5 rats. Control *Vs* test not significantly different.
*Test 1; **Test 2.

In vivo: Labelled amino acids 100 mg each (1.12, 0.58, 0.68 m moles respectively of alanine, histidine and glutamic acid) in saline administered by gavage along with 1.0 ml groundnut oil to controls and with 1.0 ml groundnut oil + 1.5 mg capsaicin/rat to test animals. Absorption (m moles)=Difference in radioactive counts/specific activity of the respective amino acid.

In vitro[a]: 5 cm everted consecutive jejunal sacs in duplicate from control and capsaicin fed rats for each amino acid (10 mM 10.51×10^5 0.89×10^5, 7.85×10^5 cpm respectively) incubated at 37°C in 10 ml Krebs-Ringer phosphate buffer pH 7.4 containing glucose (11.1 mM) for 30 min.

In vitro[b]: Everted sacs from rats fed control diet only prepared in a similar way and incubated in media with 10 mM of each amino acid (1.23×10^5, 1.28×10^5, 1.37×10^5 cpm respectively) and without or with added capsaicin 7.5 mg % in test 1 and 15.0 mg % in test 2.

TABLE 2. INFLUENCE OF CAPSAICIN ON ABSORPTION OF FAT FROM G.I. TRACT IN RATS

	G.N. oil (g)	Capsaicin (mg)	Left over fat (g)	% absorption
In presence of added capsaicin[a]				
Control (6)	1.28±0.01	—	0.76±0.04	40.8±2.84
Test (6)	1.28±0.01	1.5	0.79±0.07	38.4±2.73
Control (12)	1.35±0.03	—	1.00±0.04	25.8±2.21
Test (12)	1.35±0.01	4.5	0.85±0.05	36.8±3.55*
In capsaicin fed rats[b]				
Control (5)	1.83±0.01		0.90±0.14	50.7±5.24
Test (5)	1.83±0.01	+	0.95±0.10	48.3±3.93

[a]. Groundnut oil with or without capsaicin dissolved in it administered by gavage.

[b]. Groundnut oil alone administered to control capsaicin fed rats. Values are mean±S.E.M.

* Value Significantly different from control.

\+ Rats maintained on 15mg % capsaicin diets for 6 weeks.

experiments only in view of the limitations of *in vitro* methods[8]. There seems to be no adverse influence on absorption of groundnut oil either from G.I. tract in the presence of capsaicin or in rats maintained on diets containing the latter compound (Table 2). Earlier studies[9] in which the whole spice 'red pepper' (at 5 per cent equivalent to 15 mg per cent capsaicin) was fed along with low or high fat (15 or 30 per cent) and 5 per cent casein also did not bring about any inhibitory effect. In contrast to our observations Nopanitaya[2] reported inhibition of fat absorption in Sprague Dawley rats maintained on capsaicin containing (14 mg per cent) low protein (10 per cent casein) diets for 4 or 8 weeks. As seen from Table 2 capsaicin at a higher level seems to even enhance fat absorption. Taking into consideration the low level of human intake (250-300 mg whole spice or 0.75-0.9 mg capsaicin/person/day) it can be safely concluded that capsaicin exerts no adverse effect on either amino acid or fat absorption.

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Effect of Ivy Gourd (*Coccinia indica* Wright and Arn.) Constituents on Tinplate Can Corrosion

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The role of chemical constituents of ivy gourd on the overall corrosion of tinplate was studied. It has been shown that many constituents have synergistic action on nitrate induced corrosion. This is very predominant in the case of copper.

Chemical analysis of ivy gourd, accumulation of high levels of nitrate in the vegetable and its role on the heavy detinning have been reported earlier.[1] Nitrate induced corrosion is pH dependent which has been reported by many workers[2–5]. Stodtz, and Henry[3] found that at pH above 5.0, the corrosion is negligible

even at 800 p p m of nitrate. In canned green beans a pH of 5.4 and nitrate content of about 200 p.p.m. dissolved tin much faster than mustard green having a pH of about 4.9 to 5.0 and nitrate content of about 800 p.p.m. They explained that nitrate induced corrosion may be inhibited in the presence of other depolarisers. This explanation does not seem to be sound. Ivy gourd also has a pH of 4.8 to 5.1 and a nitrate content of about 300 p.p.m. but is a fast detinner.

The synergistic ac ion of other constituents on nitrate induced corrosion has not been studied in detail. Role of individual constituents and fractions of ivy gourd on corrosion is studied and their cumulative action evaluated. Electrochemical theory suggests that copper can accelerate nitrate induced corrosion which may explain the fast detinning of tinplate by ivy gourd eventhough its pH is around 5.0. This aspect of the study is being published separately.

Materials and Methods

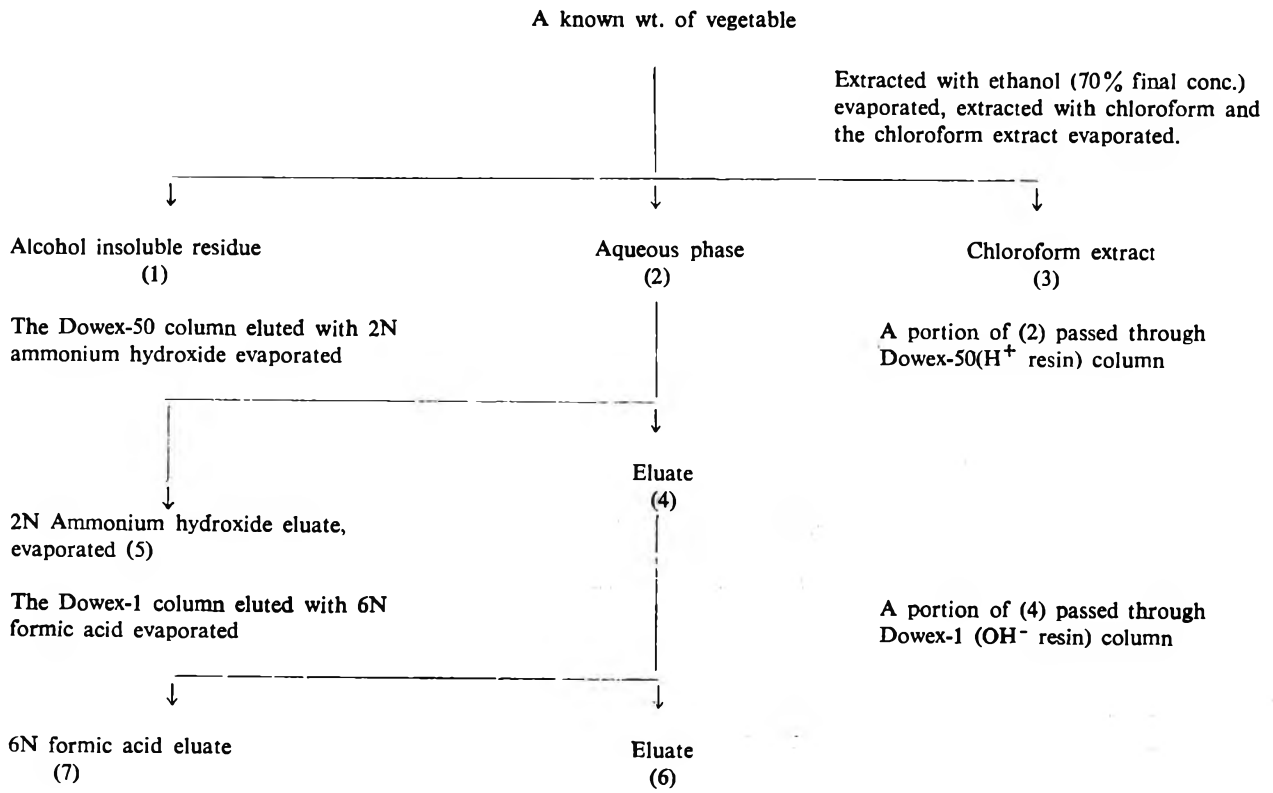
Ivy gourd: Fresh, tender ivy gourd grown near Mysore, India, was used in this study. The analysis of ivy gourd for a particular year and season was carried out on a single batch. Ivy gourd was freeze dried, powdered and stored at –18°C and required quantities were drawn whenever necessary. The analytical data were finally computed to fresh weight values.

Tinplate and cans: For most of the studies in this work, electrolytic tinplate (E-100) and cans fabricated from tinplate from a single source and batch were used. The tinplate used for the studies (unless otherwise specified) had the following characteristics:

Tinplate characteristics	Value
Tin coating weight (g/m^2)	22.4
Alloy layer (g/m^2)	1.8
Iron solution value (ISV)	8.0
Tin coating grain size (ASTM No.)	8.0
Alloy tincouple value (μ A/cm^2)	0.11
Base plate phosphorus (%)	0.02

S.M.C. buffer: Succinate-malate-citrate (S.M.C.) buffer used in this study is normally employed for corrosion studies in model experiments. It is a 0.05 N buffer having succinic, malic and citric acids in equivalent ratios of 4.1, 2.1 and 0.7 respectively which represent the ratio in which these are present in normal maturity ivy gourd. The pH of the buffer used in individual experiments are indicated in the text.

Fractionation and analysis of ivy gourd: The following scheme shows the method followed for the fractionation of the vegetable. The numerals in the parenthesis denote the fraction number which is referred to in the text.



The above fractions, except 1 and 3, were diluted or concentrated to the same level as obtained in two times diluted vegetable extract referred in canning practice. The vegetable constitutes only about 50 per cent by wt, of the content of the cans, the rest being brine.

In this corrosion study a number of other solutions were also made use of. The vegetable was blended with equal quantity of 2 per cent sodium chloride solution. This sample was coded as (8). One per cent sodium chloride solution formed test solution (9).

The pH of all the fractions and solutions except (1) and (3) were adjusted to that of (8) which for ivy gourd was 4.9 using sodium hydroxide or a mixture of succinic, malic and citric acids in the ratio of 4.1 :2.1 :0.7. All the solutions had a final sodium chloride concentration of 1 per cent. The alcohol insoluble residue (3.6 per cent of fresh material) was mixed with other solutions in some studies in such proportions as to correspond to half the original percentage.

Analysis of tinplates: Tin coating weights of the tinplates were determined by the difference in weight using antimony trichloride in concentrated hydrochloric acid[6]. Suitable correction was made for the alloy layer. Iron solution values of the tinplates used were determined by the method developed by Willey *et al*[7], which is a measure of the quantity of iron dissolved from 19.35 cm^2 tinplate into a test solution in 2 hr. The tin coating grain sizes were revealed and compared with ASTM standards[8]. For the determination of phosphorus content in the base plates, the tinplates were detinned completely before dissolving the steel in aqua-regia to prevent the retention of some portion of the phosphorus in the insoluble residue of metastannic acid. After dissolving in aqua-regia and removing the nitric acid, the phosphorus was determined by the molybdate method[9]. Methods followed for the various chemical estimations were the same as reported earlier[1]. Hydroxy methyl furfural was estimated by the method of Luh *et al*[10]. Furfural was determined by the photometric method of Amerine *et al*[11].

Corrosion studies: Test solutions (35 ml) were filled in glass tubes at 90°C and tinplate strips of 30 cm^2 surface area and known weights were immersed in the solution. The cut edges of the tinplate strips were coated with a special inert thermostable resin to fully cover the exposed steel at the edges. The tubes were boiled to expel air and then sealed by melting, cooled and stored at the required temperature. Periodically the tubes were broken and the loss in weight of the tinplates were determined. The average weight loss of four tinplate strips was taken as the loss of weight. The variation in weight loss among tinplate strips was not significant.

Measurement of vacuum: A short pressure tubing was attached to a vacuum gauge. The open end of the tubing was fixed to the sealed end of the tubes and the tip was broken without allowing air to get in. The reading on the gauge was noted.

Results and Discussion

Corrosion of tinplate in the major fractions of ivy gourd: In Table 1 the extent of corrosion of tinplate and vacuum losses caused by the different major fractions of ivy gourd and their combinations are given. From the data the following inferences could be drawn: (*a*) the fraction containing the anions is the most corrosive; (*b*) one per cent brine, the amino acids and the sugar fractions have very low corrosive action towards tinplate;

TABLE 1. RATE OF TINPLATE CORROSION AND VACUUM CHANGES CAUSED BY THE MAJOR FRACTIONS AND THEIR COMBINATIONS OF IVY GOURD

Test solution	Degree of corrosion and vacuum (inches Hg)							
	Initial		3 months		6 months		9 months	
	C	V	C	V	C	V	C	V
Alcohol extract (1)	15	14	40	14	72	14	81	13
(1)+AIR (2)	30	14	75	14	98	14	100	13
AIR	5	14	12	14	16	13	18	12
(1) passed through Dowex-50 resin (3)	10	14	23	14	50	14	62	13
(3)+AIR	18	14	35	14	61	14	78	13
2N Ammonia eluate of Dowex-50 column (4)	6	14	10	14	17	13	20	11
(4)+AIR	7	14	12	13	19	12	25	11
(3) passed through Dowex-1 resin (5)	5	14	10	13	18	12	32	9
(5)+AIR	6	14	12	13	20	11	36	8
6N formic acid eluate of Dowex-1 column (6)	10	14	20	14	22	14	45	13
(6)+AIR	18	14	40	14	71	14	95	13
Ivy gourd+2% brine (1:1 ,w/w) blended	16	14	32	14	55	14	72	13
1% brine	4	14	12	14	15	13	18	12

Storage temperature=37°C C=Degree of corrosion (% loss in weight of tin)

V=Vacuum AIR=Alcohol insoluble residue

*soon after processing and cooling.

(*c*) the sugar fraction on long storage (more than 6 months at 37°C) shows slight corrosive action. This can be due to the formation of hydroxy methyl furfural; (*d*) the alcohol insoluble residue, though by itself is least corrosive, accelerates the corrosion rate by the anionic fraction several folds, and (*e*) the alcohol extract, though it does not contain the alcohol insoluble residue is slightly more corrosive than the whole ivy gourd. The whole blended ivy gourd (equal quantity of ivy gourd and 2 per cent brine) is a viscous material. Therefore, it is natural to expect from the well known phenomenon of retardation of corrosion rate by increasing the consistency, that in this case also such a machanism may operate.

The vacuum changes during storage show that in the presence of anionic fraction, vacuum losses are neglible, and that this fraction contains depolarisers which can accept electrons. In such a system the proton reduction and the hydrogen evolution reaction are replaced by the depolariser reduction reaction. This is in agreement with the data which show that ivy gourd accumulates nitrate which is a powerful depolariser.

Corrosion in organic acids of ivy gourd: Fig. 1 shows the rate of corrosion in SMC buffer in different equivalent proportions at 0.5N concentration and at pH 4.5 and 5.0 It is evident from the figure that as the citrate concentration increases in the buffer, the degree of corrosion also increases. This is important in the canning of ivy gourd because during maturation of ivy gourd there is a steep increase in citrate concentration[1] and the citrate malate ratio is altered indicating that selection of the raw material of correct maturity becomes an important factor. The reason for the higher corrosiveness of citric acid may be duc to its ability to chelate stannous ions thereby decreasing the corrosion inhibitory power of the stannous ions. The stability constant of tin citrate

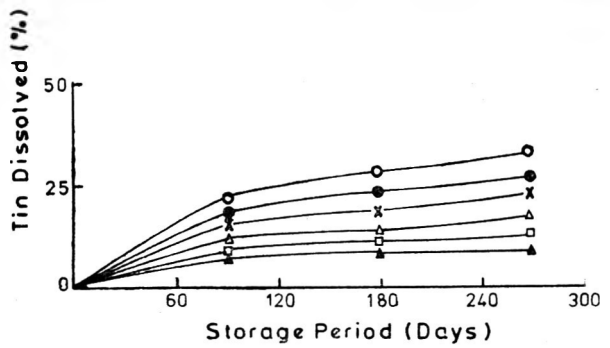


Fig. 1. Tinplate corrosion in S.M.C. buffer

O—O (3.9, 1.6 1.6 pH 4.5, ● — ● (4.1, 2,1, 0.7) pH 4.5
×—× (4.3, 2.4, 0.2) pH 4.5, △ — △ (3.9, 1.6, 1.6) pH 5.0
□—□ (4.1, 2.1, 0.7) pH 5.0, ▲ — ▲ (4,3, 2.4, 0.2) pH 5.0

The figures in parenthesis indicate equivalent concentrations of succinate, malate and citrate

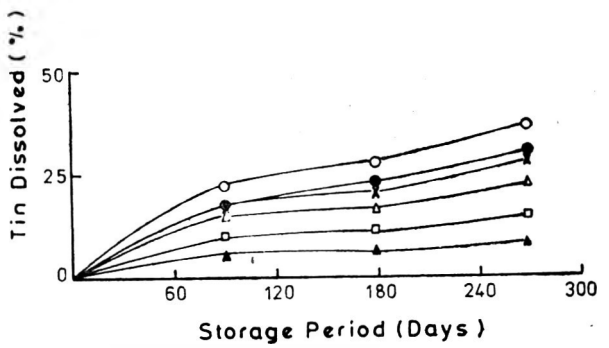


Fig. 2. Tinplate corrosion in different organic acid buffer

O—O Citric acid pH 4.5, ×—× Citric acid pH 5.0
●—● Succinic acid pH 4.5, △—△ Succinic acid pH 5.0
□—□ Malic acid pH 4.5, ▲—▲ Malic acid pH 5.0

is almost double that for tin malate complex[12]. The increase in the rate of corrosion due to a decrease in pH, though well known, is of significance in the narrow region of pH 0.5 unit because of the pH of ivy gourd is normally found to fluctuate in the region of 4.5 to 5.0.

Fig. 2 shows the effect of individual organic acids buffered to pH 4.5 and 5.0 on the rate of corrosion of tinplate. Malic acid is the least corrosive among the three acids and citric acid the most corrosive. The corrosion rate of tinplate in SMC buffer of different normalities and buffer capacities showed that there is a slight increase in corrosion rate with increase in normality.

Corrosion in sugars: Fig. 3 shows the rate of corrosion of tinplate in SMC buffer solution of pH 4.5 and 5.0 containing glucose at 0.7 per cent level. This concentration was chosen to represent almost the actual concentration of glucose in canned ivy gourd. It is evident that upto 90 days storage at 37°C, the rate of corrosion is very low and thereafter it increases rapidly. This can be assumed to be due to the formation of hydroxy methyl

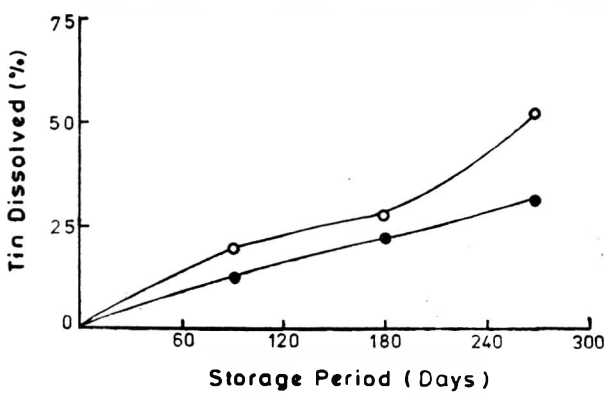


Fig. 3. Tinplate corrosion in S.M.C. buffers containing glucose

O—O pH 4.5, ●—● pH 5.0

TABLE 2. FORMATION OF HYDROXY METHYL FURFURAL AND FURFURAL IN MODEL BUFFERS CONTAINING GLUCOSE

Storage period (days)	HMF formed (ppm)		Furfural formed (ppm)	
	pH 5.0	pH 4.5	pH 5.0	pH 4.5
0	0	0	0	0
90	2	5	3	5
180	6	15	10	18
270	18	40	23	55

Storage temperature is 37°C

furfural (HMF) and furfural formed under the experimental conditions as above except in the absence of tinplate. Formation of these compounds upto 90 days is very slow and thereafter it increases. This finding explains the role of these compounds in the acceleration of tinplate corrosion.

Corrosion in amino acids: Fig. 4 shows the effect of amino acids from ivy gourd on the rate of corrosion of tinplate in S. M. C. buffer of pH 4.5 and 5.0. The concentration used was equivalent to the average concentration found in canned ivy gourd. It shows that the corrosion caused by the amino acids is not significantly more than that contributed by the buffer. Earlier[1] it has been shown that the amino acid pool is deficient in cystine or cysteine which can cause the sulphide stain. The tinplates did not show any sulphide stain formation. As the whole amino acid fraction did not show any acceleration or inhibition effect on corrosion, it is natural not to expect

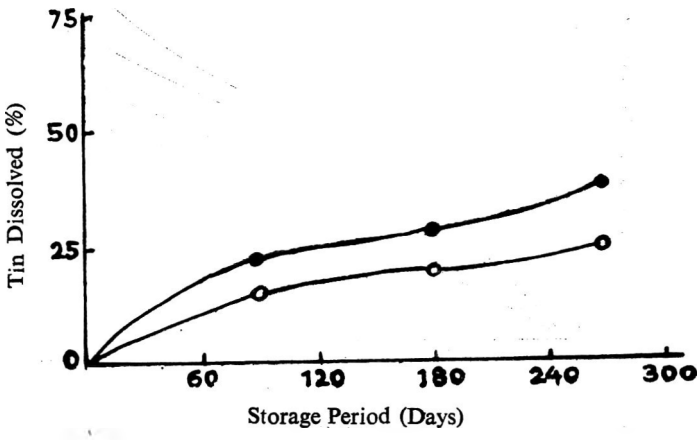


Fig. 4. Tinplate corrosion in S. M. C. buffers containing amino acids from ivy gourd.

●——● pH 5.0, O——O pH 4.5

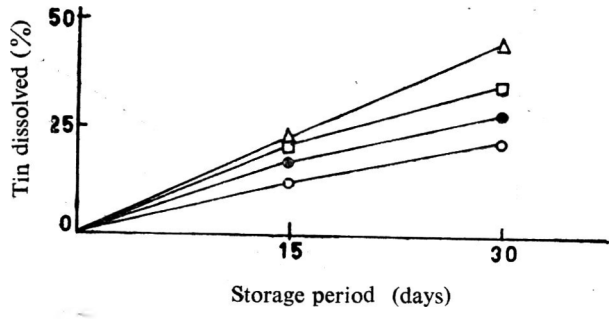


Fig. 5. Tinplate corrosion in SMC buffer containing amino acid from ivy gourd and cysteic acid.

O——O SMC Buffer pH 4.5, ●——● + Amino acids pH 4.5, □——□ + 50 μ moles Cysteic acid pH 4.5, △——△ + 100 μ moles Cysteic acid pH 4.5.

any of the individual amino acids or the three unidentified ninhydrin positive compounds to have any corrosion acceleration or inhibition properties. Cysteic acid has been reported to be formed during hydrolysis. It is possible that these compounds may be formed in larger quantities during storage. Fig. 5 indicates the role of cysteic acid in 50 and 100μ molar concentration in SMC buffer at pH 4.5 and the amino acid mixture at ten milli molar percent concentration on the corrosion of tinplate. Both at 50 and 100 μ molar concentration cysteic acid showed higher rate of corrsion.

Corrosion in nitrate: As the range of nitrate in ivy gourd shows wide variations[1] the effect of nitrate was studied at two levels, namely 50 and 200 ppm. Since nitrate induced corrosion of tinplate is highly pH dependent, this was studied at pH of 4.0, 4.5, 5.0 and 5.5 and the results are shown in Fig. 6 and 7.

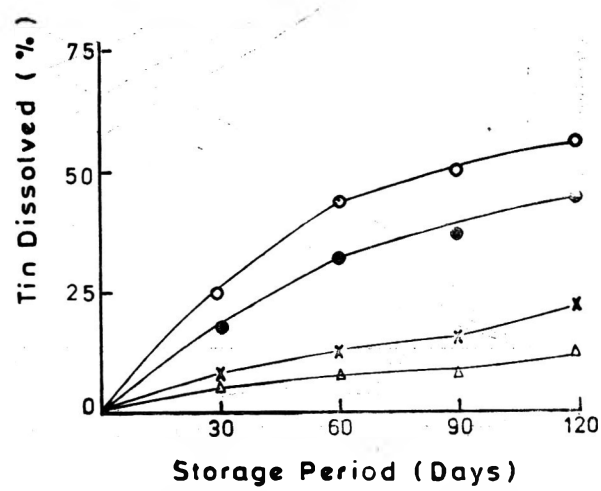


Fig. 6. Tinplate corrosion in S.M.C. buffers containing 50 ppm nitrate

O—O pH 4.0, ●—● pH 4.5, ×—× pH 5.0, △—△ pH 5.5

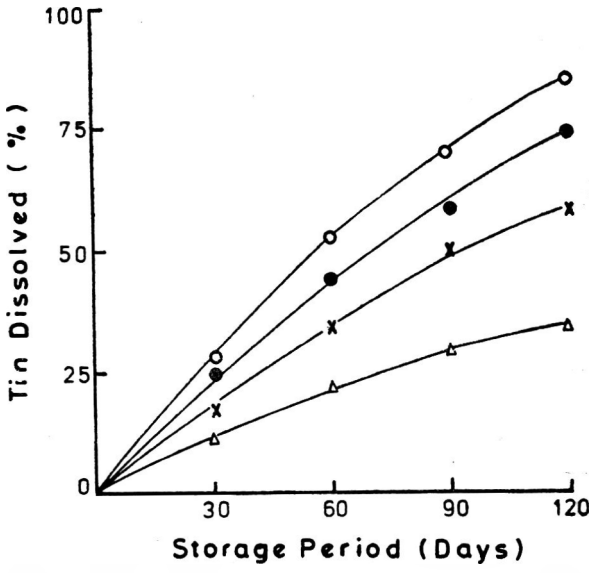


Fig. 7. Tinplate corrosion in S.M.C. buffer containing 200 ppm nitrate

O—O pH 4.0, ●—● pH 4.5, ×—× pH 5.0, △—△ pH 5.5

When the nitrate concentration increased from 50 to 200 the rate of corrosion also increased slightly. Above pH 5.0, the rate of corrosion is slow. Below pH 5.0 rate of corrosion is fast in S.M.C. buffers containing both 50 and 200 ppm nitrate.

Fig. 8 shows the inter relationship between nitrate lost and ammonia formed in S.M.C. buffer containing 200 ppm nitrate at pHs of 4.0, 4.5, 5.0 and 5.5. There is a proportionate increase in ammonia formed as nitrate is used up. However, about 2 to 5 per cent of the nitrogen could not be accounted for. This may be due to the small quantity of ammonia present in the headspace as well as due to the formation of other reduction compounds of nitrate like NO, NO_2 and N_2 in small quantities[13]

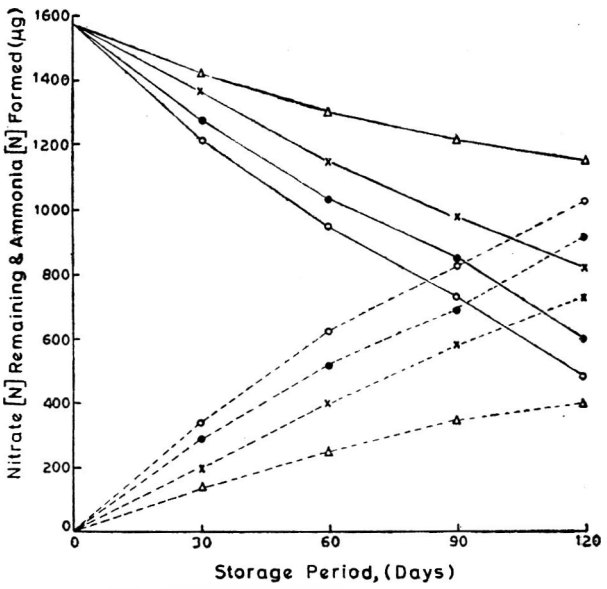


Fig. 8. Nitrate lost and ammonia formed during corrosion of tinplate in S.M.C. buffers containing 200 ppm nitrate

Nitrate remaining at: O——O pH 4.0, ●——● pH 4,5, ×——× pH 5.0, △——△ pH 5.5, Ammonia formed at: O····O pH 4.0, ●····● pH 4.5, ×····× pH 5.0, △····△ pH 5.5

It is evident from the nitrate corrosion studies of tinplate that there is a stoichometric relation between nitrate converted and tin dissolved. This indicates that at least in simple systems like the buffer used, such a relationship will hold good. From the results of tinplate corrosion studies with constituents from ivy gourd so far presented, it is evident that nitrate is the compound which is essentially the corrosion accelerator. The other constituents contribute little towards tinplate corrosion, individually.

Corrosion in organic acids and nitrate: Succinic, malic and citric acids were studied for their corrosiveness in the presence of nitrate at different pH. They did not show any effect on nitrate induced corrosion of tinplate.

Corrosion in glucose and nitrate: The cumulative effect of glucsoe at 0.7 per cent level and nitrate at 100 p.p.m. level in S.M.C. buffer of pH 4.5 on the corrosion of tinplate was studied. During the first 120 days of storage, glucose did not change the rate of corrosion caused by nitrate. At the end of 120 days all the tin had dissolved. By repeating the experiment with 50 ppm nitrate and 0.7 per cent glucose it was possible to determine the rate of corrosion upto 270 days (Fig. 9).

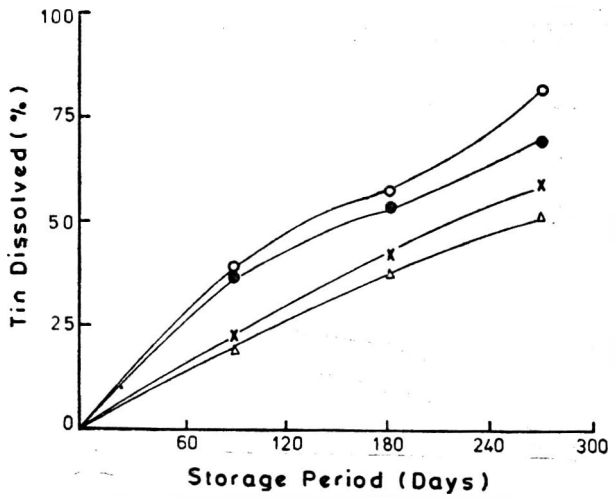


Fig. 9. Tinplate corrosion in S.M.C. buffers containing nitrate (n) and glucose (g)

O——O (n) + (g) pH 4.5, ●——● (n) pH 4.5
×——× (n) + (g) pH 5.0, △——△ (n) pH 5.0

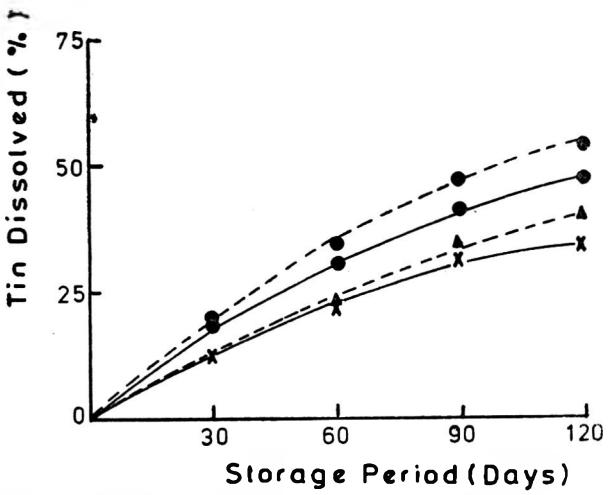


Fig. 10, Tinplate corrosion in S.M.C. buffer containing nitrate (n) and amino acids (AA) from Ivy gourd

●—●(n) pH 4.5, ●····●(n)+(AA) pH 4.5,
Δ····Δ (n) pH 5.0, ×——×(n)+(AA) pH 4.5

The accelerating action of glucose on the nitrate induced corrosion after 180 days is more evident in the system having a pH value of 4.5. These results confirm the formation of hydroxy methyl furfural.

Corrosion in amino acids and nitrate: The amino acid mixture isolated from ivy gourd was studied for its effect on nitrate induced corrosion at 1 milli mole per 100 ml level in S.M.C. buffers of pH 4.5 and 5.0 containing 100 ppm nitrate (Fig. 10). There is only a slight cumulative corrosion action due to the amino acids.

Acknowledgement

Authors thank Dr. M. V. Patwardhan for his valuable suggestions and Dr. B. L. Amla, for his keen interest in this investigation.

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Role of Traces of Cupric Copper on Nitrate Induced Corrosion— A New Mechanism of Nitrate Induced Corrosion

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Traces of cupric copper has been demonstrated to accelerate several folds, nitrate induced corrosion. Based on electro chemical and thermodynamic considerations, mechanism for the nitrate induced corrosion of tinplate has been proposed. This explains the extensive detinning property of some vegetables having pH of about 5.0, which is not favourable to nitrate induced corrosion.

In earlier communications the chemical composition of ivy gourd, accumulation of nitrate by the vegetable and the role of chemical constituents on overall corrosion of tinplate with particular reference to nitrate induced corrosion have been reported[1,2]. The alcohol insoluble residue was found to exert the maximum synergistic action on nitrate induced corrosion among the constituents of ivy gourd[2]. In this paper the results of corrosion studies on the various components of alcohol insoluble residue are presented.

Materials and Methods

Ivy gourd and tinplate used in this experiment were similar to those reported earlier[2]. Alcohol insoluble residue from ivy gourd was prepared and fractionated as described in the earlier report[1]. Copper was determined by the method of Ramsey[3]. The valency state of copper was determined by 2,2-biquinoline method[4]. Iron was determined by the thiocyanate method of Wong[5]. Crude fibre and protein were determined by AOAC methods[6]. Molybdenum was determined by dithiol method[7]. Pectin was estimated by the carbozole galacturonic acid reaction method[8]. Corrosion studies in sealed glass tubes were carried out as described in an earlier communication[2].

Results and Discussion

The proximate composition ot the alcohol insoluble residue is given in Table 1.

TABLR 1. PROXIMATE COMPOSITION OF THE ALCOHOL INSOLUBLE RESIDUE (DRY WEIGHT BASIS)

Component	Per cent
Pectin	35.2
Protein	24.4
Crude fibre	40.2

The effect of alcohol insolube residue on nitrate induced corrosion was studied at a 2 percent level and 100 ppm nitrate in Succinate-Malate-Citrate (S.M.C.) buffers of pH 4.0, 4.5, 5.0 and 5.5. It was found that the alcohol insoluble residue has a profound synergistic action on nitrate induced corrosion. The rate of corrosion was four times more at pH 4.0, 4.5 and about twice at pH 5.0 and 5.5 in the presence of alcohol insoluble residue. The corrosion of tinplate at pH 5.5 is faster (Fig. 1) when the alcohol insoluble residue is added to nitrate corroding system. This, to some extent explains

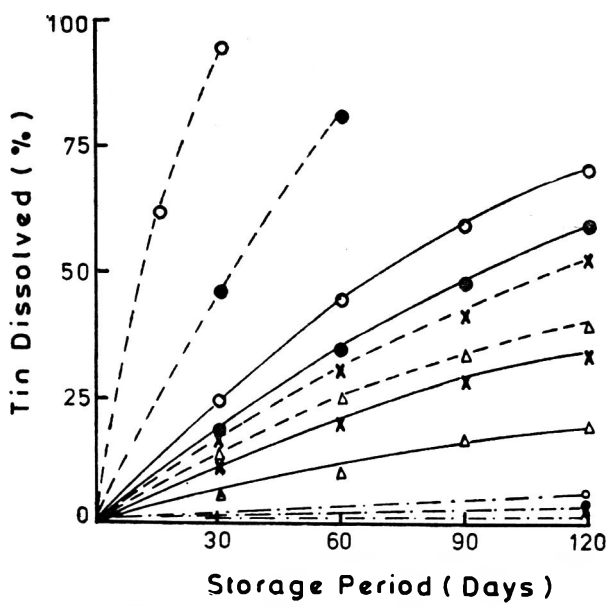

Fig. 1. Tinplate Corrosion in S.M.C. buffers containing nitrate (n) and alcohol insoluble residue (AIR)

O——O (n) pH 4.0; O... O (n) + (AIR) PH 4.0, ●—● (n) pH 4.5, ●....● (n) + (AIR) pH 4.5, ×——× (n) pH 5.0, ×....× (n) + (AIR) 5.0, △——△ (n) pH 5.5, △....△ (n) + (AIR) pH 5.5, O—.—O (AIR) pH 4.0, & 4.5 ●—.—● (AIR) pH 5.0, ×—.—× (AIR) pH 5.5

the observations made on canned ivy gourd where corrosion was noticed even above pH 5.0. This also explains the reason for detinned patches noticed whenever ivy gourd is in close contact with the tinplate[2]. The residue which cannot go into solution completely, may accelerate the nitrate induced corrosion under these conditions in conjunction with variation in pH. This observation led to the study of the role played by the constituents of the alcohol insoluble residue towards corrosion of tinplate in the presence of nitrate.

A study of the effects of the pectin, protein and crude fibre fractions at concentrations which represent the original 2 per cent of the alcohol insoluble residue in presence of 100 ppm nitrate in S.M.C. buffer at different pH values showed that the protein fraction is mainly responsible for the synergistic action on nitrate induced corrosion of tinplate. The influence of pectin and crude fibre fractions is negligible. (Fig. 2). It is unsual for protein to cause corrosion. Perhaps the protein enzyme containing one or more metals forming the prosthetic group causes corrosion.

Copper in the soluble and insoluble form: The analysis of ivy gourd for copper indicated that 29 per cent was present in soluble form whereas 71 per cent in insoluble form. It is probable that during processing this proportion will get altered and more of the copper will get solubilized as the protein which are partly enzymic in nature will be leached into the liquid medium.

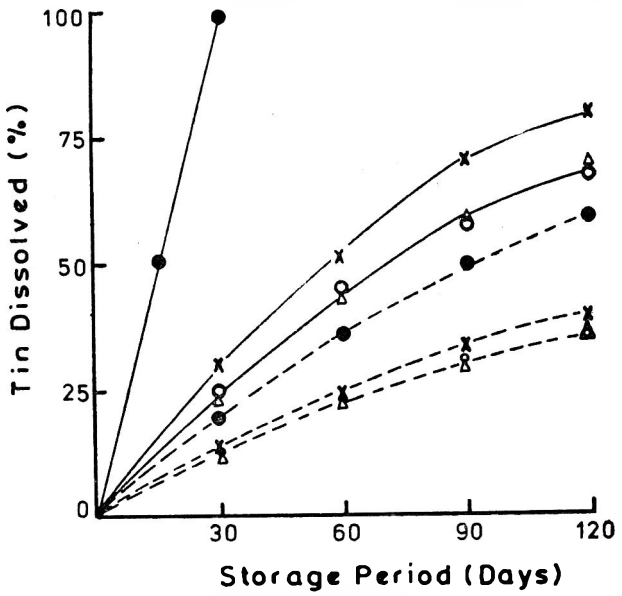

Fig. 2. Tinplate corrosion in S.M.C buffers containing nitrate (n) and fractions of AIR viz., protein (Pr), Pectin (Pe) and crude fibre (Cf).

O—O (n) pH 4.0, ●—● (n) + (Pr) pH 4.0, ×—× (n) + (Pe) pH 4.0, △—△ (n) + (Cf) pH 4.0, O ...O (n) pH 5.0, ●....● (n) + (Pr) pH 5.0, ×....× (n) + (Pe) pH 5.0, △....△ (n) + (Cf) pH 5.0

It was observed that on pressure processing (as happens in canning), the copper content in the soluble form is increased to 52 per cent. This change is very important for nitrate induced corrosion.

The valency state of copper in ivy gourd: The valency state of copper in fresh and pressure processed ivy gourd as determined by 2', 2'—biquinoline showed that in both the cases about 70 per cent of the copper is in the cupric form. Eventhough, electron magnetic resonance spectroscopy gives exact valency state of metals, the value obtained by us also indicate the importance of the valency state of copper in the corrosion of tinplate.

Analysis of the protein fraction showed that it contained 8 mg copper per 100 g wnile iron and molybdenum were present in traces. Therefore cupric sulphate was taekn for corrosion studies along with nitrate in pH 4.5 and 5.0 buffer.

Synergistic effect of copper in nitrate induced corrosion and a new theory of nitrate corrosion: Fig. 3 shows the effect of added copper as copper sulphate on nitrate induced corrosion (100 ppm nitrate) of tinplate in S.M.C. buffer of pH 4.5 and 5.0. Several concentrations of copper were tested and the results are presented in the figure, as ppm copper versus corrosion rate (weight loss during 90 days). At pH 4.5 and 2 ppm copper level the rate of corrosion was very high, while at pH 5.0 more than 5 ppm of copper was required to increase the corrosion rate significantly. At both pH values, copper in the absence of nitrate did not have any corrosion acceleration effect even upto 10 ppm level.

Most of the cuprous salts when added to aqueous solution get decomposed to insoluble oxide. This may be the reason for cuprous salts not showing any syner-

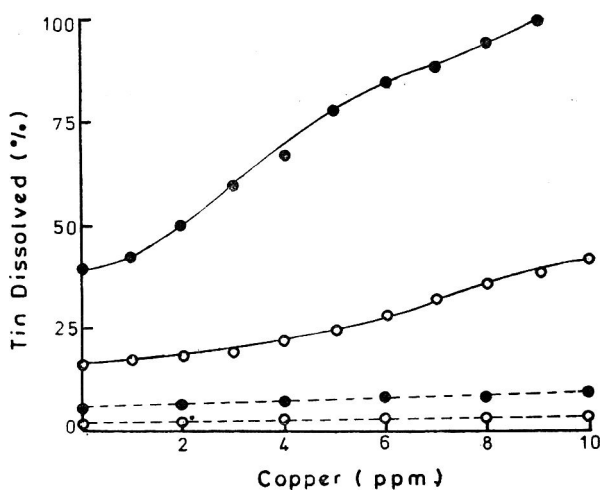

Fig. 3. Tinplate corrosion in S. M. C. buffers containing nitrate and copper

●....● pH 4.5, ●—● Nitrate + Copper pH 4.5, O....O pH 5.0, O—O Nitrate + Copper pH 5.0.

gistic effect when added to nitrate corroding system. Fom the electrode potentials of the couples involving cupric ions, cuprous ions and metallic copper, it follows that reduction of cupric ions to the metal requires far less energy than its reduction to cuprous ions.

$Cu^{+} + e^{-} \rightleftharpoons Cu$; $E^{\circ} = +0.280V$ Vs. S.C.E.
$Cu^{2+} + 2e^{-} \rightleftharpoons Cu$; $E^{\circ} = +0.103V$ Vs. S.C.E.
$Cu^{2+} + e^{-} \rightleftharpoons Cu^{+}$; $E^{\circ} = -0.074V$ Vs. S.C.E.

The quantity of copper in the soluble and insoluble form and its valency states have already been given. The results showed that about 70 per cent of the copper in ivy gourd is in cupric form. This is of considerable importance for the overall tinplate corrosion by ivy gourd.

The reason for acceleration of nitrate induced corrosion by traces of copper is difficult to explain. Probably cupric copper cannot directly take part in the initial rate limiting reaction (reduction of nitrate to nitrite) due to its higher valency state. However, an attempt has been made to explain the effective nature of copper ions in accelerating nitrate induced corrosion.

In the normal tinplate the following reactions are very slow due to the higher hydrogen overpotential of tin.

$$Sn \rightarrow Sn^{2+} + 2e^{-} \qquad (1)$$
$$2H^{+} + 2e^{-} \rightarrow 2H \rightarrow H_2 \qquad (2)$$

When cupric ions are present, it easily accepts the electrons formed in reaction (1) and get plated on to the tin surface as metallic copper.

$$Cu^{2+} + 2e^{-} \rightarrow Cu$$

Tnis brings about a decrease in the hydrogen overpotential of tin which accelerates reactions (1) and (2). Thus:

$$2H^{+} + Sn + Cu^{2+} + 2e^{-} \xrightarrow{\text{fast}} H_2 + Sn^{2+} + Cu$$

once the highly reducing stannous ions are formed, they reduce nitrate to nitrite which becomes a fast reaction.

$$2NO_3^{-} + Sn^{2+} \xrightarrow{\text{fast}} 2NO_2^{-} + Sn$$

and once the nitrite ions are formed, the other fast reduction reaction follow which facilitate the removal of the electrons formed in reaction (1) causing accelerated tin dissolution

$$NO_2^{-} + 6e^{-} + 8H^{+} \xrightarrow{\text{fast}} NH_4^{+} + H_2O$$

Copper thus appears to have a triggering action in the nitrate induced corrosion of tinplate

Acknowledgement

The authors thank Dr. M. V. Patwardhan for his valuable suggestions and Dr. B. L. Amla, for his keen interest in this investigation.

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Effect of Packaging on Quality of *Sohan Halwa* During Storage

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***Sohan halwa*, an Indian sweetmeat, based on germinated wheat flour, has a short shelf life of about 15-20 days under normal conditions. To extend the storage life, *Sohan halwa* was treated with BHA and packed separately in flexible pouches (aluminium foil, HDPE and LDPE) and rigid container (tagger top cans) and stored at 27°C and 65% RH upto a period of 180 and 300 days respectively. The sorption-isotherm studies revealed that the product has a permissible moisture pick up of 1.7% with a critical moisture content of 3% and ERH of 25%. Chemical parameters such as free fatty acids and peroxide value were periodically estimated. Sensory evaluation indicated that the product could not be stored even for 30 days in LDPE at ambient conditions, while it can be stored for 120 days and 180 days in HDPE and aluminium foil respectively and 300 days in Tagger top cans.**

Sohan halwa, a popular Indian sweetmeat, is made of germinated wheat flour, *maida* (refined wheat flour), sugar and vanaspati (hydrogenated fat) in the proportion of 1:3:9:18. The proportion of ingredients used and method of preparation vary depending upon the sweet maker[1]. The steps involved in general are, (*i*) germinating, drying and grinding the wheat; (*ii*) mixing with *maida*, adding water and straining through a cloth; (*iii*) heating the filtrate with continuous stirring, adding sugar and some quantity of vanaspati; (*iv*) adding the remaining vanaspati gradually; and (*v*) pouring the viscous mass into moulds. After cooling and solidification, the product is removed and stored conventionally in glass cases under ambient conditions.

According to Date *et al*[2], the Indian sweets have a poor shelf life of a few days. The sweet makers feel that the product could not be stored for more than 15-20 days under normal conditions due to discolouration, development of rancid flavour and fat bloom.

Of late, because of the increase in exports of sweetmeats from India specially to Middle East, the problems of packaging and storage to ensure longer shelf life have assumed importance. An investigation was therefore carried out to study the effect of antioxidant and packaging materials on shelf life of the product during storage and the results are presented in this paper.

Materials and Methods

Sohan halwa: A leading local sweetmaker prepared *Sohan halwa* under our supervision which was used in this study. Butylated hydroxy anisole (BHA) (0.02 per cent) and citric acid (0.004 per cent) on fat basis were added and mixed well before pouring into the moulds. The product was poured into the circular stainless steel mould (3.7×1.5 cm thickness) and allowed to solidify. After cooling and solidification, the product was taken out of the mould and used for packaging and storage studies.

Moisture-sorption characteristics: To study the influence of moisture and to select appropriate packaging material to obtain desired shelf life of the product[3], the humidity-moisture relationship of the product was studied at 27°C by exposing weighed quantities of the samples in petri dishes to relative humidities ranging from 11 to 92 per cent using appropriate saturated salt solutions[4]. The samples were periodically weighed till they attained constant weight or showed signs of fungal growth. Product examination was conducted on equilibrated samples to fix the critical moisture content of the product.

Packaging materials and storage conditions: Considering the desired shelf life and the protection required

Packaging material	WVTR g/m² at 38°C and 90% RH gradient
High density polyethylene-300 G (HDPE)	1.4
Low density polyethylene-300 G (LDPE)	4.1
Laminate of 60 GSM paper/0.02 mm aluminium foil/150 G low density polyethylene (aluminium foil I)	Nil
Laminate of 60 GSM paper/150 G low density polyethylene /0.009 mm aluminium foil/ 150 G low density polyethylene (aluminium foil II)	Nil

by the product, above mentioned packaging materials were selected and their water vapour transmission rates (WVTR) were determined according to the ISI method[5].

About 100 g of the product were packed in flexible pouches as well as in rigid containers. Pouches of the size 5.0×5.5 cm of base HPDE and LDPE and 6.0×6.5 cm of two types of aluminium foil laminates were used for packaging and storage studies. The product was initially wrapped with PT cellophane-300 and later put into these pouches and heat sealed. For vacuum packaging of aluminium foil II, the filled pouch was placed in Cryovac vacuum chamber machine (Model 216-mark II) and vacuum of 25 in of Hg was obtained before sealing. Aluminium foil II package variable was included in the study to test its performance over aluminium foil I pack only from the physico-chemical point of view.

With a view to export the product, the rigid tagger top tin cans were selected for packaging. The size, internal finish and tin coating weight of the rigid container are 77×60mm, plain finish and 22.4 g/Sq. m (E-100) respectively. Among the cans used 1/3rd were flushed with N_2 (single flushing), another 1/3rd cans with CO_2 (single flushing) under 25 in vacuum and the remaining 1/3rd were as it is (plain air-no CO_2 or N_2).

All the flexible and rigid unit packages were stored at 27°C and 65 per cent RH. *Sohan halwa* packed in cans under CO_2 atmosphere and stored in refrigerator was used as control. The flexible packages were stored for 180 days and samples were withdrawn once in 30 days and rigid containers were stored for 300 days and samples were drawn at every 60 days interval for analysis.

Chemical analysis: The samples were analysed for moisture; peroxide value (PV)[6] and free fatty acid (FFA)[6] content were analysed using the fat obtained by cold extraction with chloroform. Moisture was estimated at 70°C for 24 hr under vacuum.

Sensory evaluation: The sensory evaluation sessions were carried out under ISI[7] recommended laboratory set up. A descriminative-communicative panel of 20 staff members of the Institute ranked the samples for individual quality attributes-colour and appearance, texture (finger feel and mouthfeel) and flavour (aroma and taste) by following a quality description (Table 1) developed during the panel orientation sessions. The panel also judged the acceptability of the samples. The ranked data were analysed by Kramer's rank sum procedure[3] and also reranked to compare any two treatments. The sample in aluminium foil II pack was not included for sensory evaluation.

Results and Discussion

Moisture-sorption studies: The equilibrium moisture content and relative humidity relationships are shown in Fig. 1. *Sohan halwa* with an initial moisture content of 1.4 per cent on dry weight basis equilibrates to an RH of about 12 per cent. The sorption results also indicate that the product, when equilibrated to 32 per cent corresponding to a moisture content of 3.8 per cent, lost the desirable texture. The product with a moisture content of 2.5 per cent corresponding to an RH of 22 per cent was found to have just desirable texture. Hence a moisture content of about 3.0 per cent which equilibrates to about 25 per cent RH could be considered as critical for *Sohan halwa*. Thus the product had a fairly low tolerance for moisture, a permissible moisture uptake of about 1.6 per cent. At about 60 per cent RH, the product picked up moisture rapidly which indicates capillay condensation.

TABLE 1. QUALITY DESCRIPTION OF *SOHAN HALWA*

Quality	Desirable	Undesirable
Colour and appearance	Uniform pale brown to deep brown; fairly glossy; slight fat bloom.	Dark, greyish, non-uniform; loss of glossiness/dull; heavy fat bloom.
Texture and Fingerfeel	Slightly sticky; slightly oily.	Pasty/sticky; too oily.
Mouthfeel (on chewing)	Hard; fracturing into uniform crystalline powdering; easily melting; residually very slightly sticky; slightly oily; not gritty.	Soft, powdery, pasty; residually sticky and chewy; too much of oily feel; slightly gritty.
Flavour	Fresh 'ghee' like aroma; pleasant caramalised aroma; sweet taste; clean after taste.	Loss of freshness/stale/waxy/rancid; strong caramalised/burnt aroma; off flavour/unpleasant after taste.

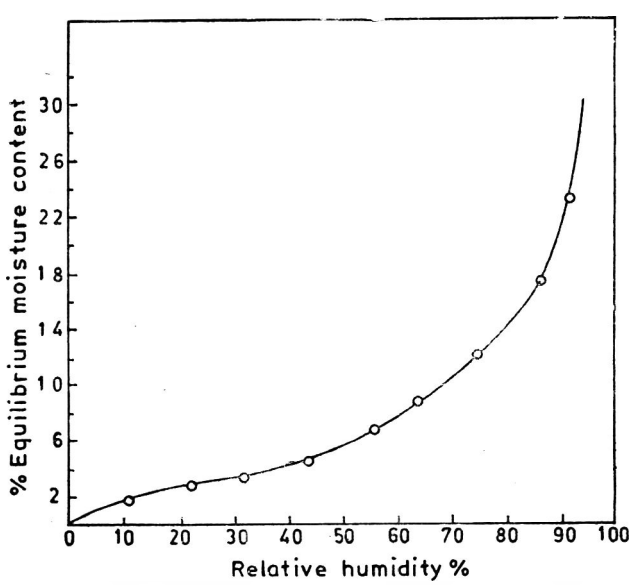

Fig. 1. Sorption isotherm for *Sohan halwa* at 27°C

TABLE 2. CHEMICAL ANALYSIS OF *SOHAN HALWA* STORED IN FLEXIBLE POUCHES

Packaging material	Storage period (days)	FFA (% oleic acid)	PV (meq/kg of fat)	Moisture (%)
	Initial	0.12	Nil	1.40
Al. foil (I)	30	0.30	Nil	1.40
Al. foil (II)	30	0.20	Nil	1.40
LDPE	30	0.41	1.0	1.73
HDPE	30	0.38	Nil	1.53
Al. foil (I)	60	0.31	Nil	1.40
Al. foil (II)	60	0.20	Nil	1.40
LDPE	60	0.60	2.0	2.05
HDPE	60	0.48	0.2	1.65
Al. foil (I)	90	0.36	Nil	1.40
Al. foil (II)	90	0.20	Nil	1.40
LDPE	90	0.72	2.2	2.08
HDPE	90	0.53	0.9	1.73
Al. foil (I)	120	0.33	Nil	1.40
Al. foil (II)	120	0.25	0.1	1.40
LDPE	120	0.74	2.5	2.53
HDPE	120	0.58	1.0	1.80
Al. foil (I)	150	0.38	Nil	1.40
Al. foil (II)	150	0.25	0.1	1.40
LDPE	150	0.91	2.9	2.56
HDPE	150	0.60	1.2	1.81
Al. foil (I)	180	0.41	Nil	1.40
Al. foil (II)	180	0.29	0.2	1.40
LDPE	180	1.00	3.0	2.69
HDPE	180	0.61	1.5	1.82

FFA=Free fatty acid PV=Peroxide value

TABLE 3. CHEMICAL ANALYSIS OF *SOHAN HALWA* STORED IN RIGID CONTAINERS

Type of Tagger top cans	Storage period (days)	FFA (% oleic acid)	PV (meq/kg of fat)
	Initial	0.12	Nil
Plain	60	0.21	Nil
N_2	60	0.20	Nil
CO_2	60	0.20	Nil
Plain	120	0.25	Nil
N_2	120	0.22	Nil
CO_2	120	0.22	Nil
Plain	180	0.26	Nil
N_2	180	0.21	Nil
CO_2	180	0.20	Nil
Plain	240	0.34	0.2
N_2	240	0.24	Nil
CO_2	240	0.23	Nil
Plain	300	0.40	0.8
N_2	300	0.30	Nil
CO_2	300	0.30	Nil

FFA=Free fatty acid PV=peroxide value

The flexible packages exposed to storage conditions of 27°C and 65 per cent RH were weighed at regular intervals to follow the pick up of moisture. It can be seen from the Table 2 that even at the end of 180 days of storage period, the permissible moisture pick up of 1.6 per cent did not reach in any package. In HDPE and LDPE pouches, the actual moisture pick up was 0.42 per cent and 1.29 per cent respectively, while in aluminium foil laminate pouches the moisture content remained almost constant.

The rigid containers, also exposed to 27°C and 65 per cent RH, were examined for the condition of the cans. The can interior was normal and free from feathering, detinned spots or rusting.

Chemical analysis: The FFA and PV values analysed for the products packed in different packaging materials are presented in Tables 2 and 3.

Slight increase of FFA in sample packed in LDPE was noticed. This was followed by sample packed in HDPE. The increase of FFA in samples packed in both the aluminium foils (I & II) is not significant.

There was negligible increase of FFA in samples packed in rigid containers.

Among the samples packed in flexible packages, sample packed in LDPE showed gradual increase in PV from nil to 3.0 meq/kg of fat at the end of 180 days of storage period, while the sample packed in HDPE recorded PV of 1.5 meq/kg of fat. The sample packed in aluminium foil (I) did not give PV at all while that in aluminium foil (II) showed very insignificant changes.

In the samples packed in tagger top cans (plain), PV increased from nil to 0.8 meq/kg at the end of 300 days whereas PV of the samples packed under N_2 or CO_2 was nil throughout.

Sensory evaluation: The results of periodical sensory evaluation studies of the product in different packages are presented in Table 4. LDPE showed unsatisfactory performance of the product even after 30 days of storage. The sample packed in LDPE was found to be unsatisfactory in colour, appearance and flavour.

The performance of HDPE was found to be satisfactory upto 120 days storage. The product packed in aluminium foil was comparable to control in quality and acceptability throughout the storage period of 180

TABLE 4. RANK SUM ANALYSIS OF INDIVIDUAL QUALITY AND PER CENT ACCEPTABILITY OF *SOHAN HALWA* SAMPLES

Flexible pouches					Rigid containers				
Storage period (days)	LDPE	HDPE	Al. foil I	Control	Storage period (days)	TT plain	TT CO_2	TT N_2	Control
				Colour and appearance					
30	80^b	50^a	35^a	35^a					
60	78^b	45^a	41^a	36^a	60	57	50	52	41
90	65^d	51^c	45^b	39^a	120	59	52	50	39
120	71^d	54^c	45^b	30^a	180	61	56	44	39
150	58^b	56^b	55^b	31^a	240	60	53	44	43
180	60^c	59^{bc}	45^{ab}	36^a	300	57	53	46	44
				Fingerfeel					
30	77^b	53^a	36^a	34^a					
60	74^c	53^b	44^b	29^a	60	53	51	49	47
90	77^b	39^a	44^a	40^a	120	60	50	46	44
120	74^c	57^{bc}	40^{ab}	29^a	180	60	49	47	44
150	65^b	60^b	48^{ab}	32^a	240	55	53	49	43
180	74^c	50^b	44^{ab}	32^a	300	56	51	48	45
				Mouthfeel					
30	78^b	48^a	42^a	32^a					
60	74^c	53^b	46^b	27^a	60	51	53	51	45
90	60^b	49^{ab}	56^{ab}	35^a	120	51	48	48	43
120	70^c	55^b	52^b	23^a	180	61	50	49	40
150	64^c	57^{bc}	44^{ab}	35^a	240	61	55	45	39
180	70^d	58^c	46^b	26^a	300	60	54	46	40
				Flavour					
30	78^c	52^b	36^{ab}	34^a					
60	74^c	46^b	54^b	26^a	60	58	50	50	42
90	69^c	52^b	50^b	29^a	120	56	51	50	43
120	67^c	57^{bc}	51^b	25^a	180	54	52	50	44
150	63^b	59^b	38^a	40^a	240	55	53	50	42
180	71^c	60^b	40^a	29^a	300	59	50	45	42
				% Acceptability					
30	50	90	95	100					
60	45	80	90	100	60	90	95	100	100
90	40	75	85	100	120	90	95	95	100
120	40	75	85	100	180	85	95	95	100
150	35	55	85	95	240	85	90	90	100
180	35	55	80	95	300	80	90	90	95

TT: Tagger Top: Under flexible pouches rank sums carrying different superscripts in the same row differ significantly ($P \leq 0.05$). Under rigid containers rank sums in each row do not differ significantly ($P \leq 0.05$)

days and this pack may also be considered for exporting the product. Though sample in aluminium foil II pack was not included for sensory evaluation, a better performance of this package variable could be expected as sample in aluminium foil I package variable has shown good shelf life.

The performance of the product packed in rigid containers with and without N_2 and CO_2 gas packing

was found to be satisfactory upto 300 days of storage period.

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RESEARCH NOTES

CHEMICAL COMPOSITION AND *IN VITRO* EVALUATION OF PROTEIN QUALITY OF MAIZE KERNELS AND THEIR PRODUCTS

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Chemical composition and protein quality based on lysine, tryptophan and *in vitro* protein digestibility of various dry mill maize products were evaluated and compared with whole kernel. In these products, protein content varied from 8 to 11.5, ash from 0.4 to 6.48, fat from 0.84 to 6.85 and crude fibre from 0.13 to 4.08 per cent. Germ was the richest source of fat, protein, ash and crude fibre. The lysine and tryptophan contents were higher in germ, *maida* (refined flour) and mill *atta*, whereas *in vitro* protein digestibility was found to be in the range of 75 to 80 per cent in whole kernel, corn flakes, *sooji*, grits, mill *atta* and precooked flour.

In India, maize is consumed as food in different forms. Dry milling process produces different products some of which are put to various food uses. Present investigation was undertaken to study the chemical composition and protein quality of various dry milled products with reference to whole kernel.

Protein, ash, fat and crude fibre in the different dry mill maize products obtained from Karnataka State Agro-Corn Product Ltd., Bangalore, were determined according to standard methods[1]. Lysine and tryptophan in defatted samples were estimated by colorimetric methods[2,3]. *In vitro* protein digestibility (IVPD) was estimated by the procedure of Saunders and Kohler[4] with slight modification using pronase and trypsin. Calculation was done as follows:

$$\text{IVPD in precipitate} = \frac{\text{N in sample-N in precipitate}}{\text{N in sample}} \times 100$$

$$\text{IVPD in supernatant} = \frac{\text{N in supernatant}}{\text{N in sample}} \times 100$$

Means of both the values were used.

Crude protein in the germ was the highest being 2.36 times that in whole kernel while in flakes it was slightly lower than that in the whole kernel (Table 1). Ash content in flakes was nearly half of that found in whole kernel, while it was 4.5 times more in the germ. Compared to whole kernel, fat content was very high (34 per cent) in germ and very low (less than 1 per cent) in flakes. In various mill products, namely, *sooji*, *maida*, mill *atta*, precooked *sooji* and precooked flour, which are supple-

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TABLE 1. CHEMICAL COMPOSITION OF DIFFERENT MAIZE PRODUCTS*

Maize products	Protein %	Ash %	Fat %	(Crude fibre) %
	Yellow maize			
Whole kernel	9.91	1.27	5.01	3.48
Mill *atta*	9.95	2.52	5.00	0.13
Germ	23.41	5.68	34.19	7.24
Flakes	9.13	0.42	0.90	0.15
Grits	9.61	0.91	3.30	0.34
Sooji	9.73	0.87	5.18	0.73
	White maize			
Whole kernel	10.16	1.76	5.08	2.59
Precooked flour	9.27	0.83	2.12	0 51
Flakes	9.42	0.46	0.84	0 26
Maida	9.46	2.18	5.27	0.26
Precooked *sooji*	8.30	0.41	1.02	0.39

*Mean of duplicate analysis, on dry wt. basis.

TABLE 2. LYSINE, TRYPTOPHAN AND *IN VITRO* PROTEIN DIGESTIBILITY OF DIFFERENT MAIZE PRODUCTS*

Maize products	Lysine (g/16g N)	Tryptophan (g/16g N)	Chemical score** (%)	*In vitro* protein digestibility (%)
		Yellow maize		
Whole kernel	2.93	0.33	53	75.10±0.76
Mill *atta*	3.40	0.60	62	78.00±0.95
Germ	5.43	0.77	99	67.36±0.43
Flakes	1.87	0.29	34	80.87±0.41
Grits	2.19	0.38	40	79.05±2.10
Sooji	2.13	—	39	78.13±0.93
		White maize		
Whole kernel	2.00	0.28	36	77.51±0.33
Precooked flour	2.65	0.36	48	76.65±0.49
Flakes	1.93	0.28	35	79.18±0.35
Maida	3.66	0.61	67	63.91±1.87
Precooked *sooji*	1.83	0.27	33	77.37±0.62

*Mean of duplicate analysis.

**Based on lysine as the first limiting amino acid and 5.5 g lysine/16 g N amino acid pattern[8].

±SE of mean of triplicate analysis.

mentary and preparatory mill products, the protein content did not vary much and ranged from 8.3 to 9.95 per cent. However, these products varied in the contents of ash, fat and crude fibre. Mill *atta* and *maida* were rich in fat and ash contents compared to other products. Flakes and pre-cooked *sooji* had low content of fat and ash. Among the various products flakes had lowest content of crude fibre.

Compared to whole kernel, lysine level was found to be lower in flakes, precooked *sooji* and grits, whereas it was higher in germ, *maida*, mill *atta* and precooked flour (Table 2). Higher lysine content was more pronounced in germ (about 85 per cent) whereas in mill *atta* and precooked flour it was about 16 and 32 per cent higher respectively. Similar trend was also observed for tryptophan content and chemical score. However, the IVPD in flakes, *sooji*, grits and mill *atta* was slightly higher and in germ and *maida* it was lower compared to the whole kernel. Lower IVPD in germ is due to higher crude fibre and hemicellulose content whereas in *maida*, it was due to higher hemicellulose content[5,6]. Hemicellulose content was nearly two and half fold higher in germ and maida than the level in whole kernel[7].

The study thus indicated that the maize products like *maida*, mill *atta* and precooked flour, derived from whole kernel, had superior protein quality and higher ash and fat compared to other products like flakes, grits and *sooji*, which are derived from the endosperm.

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EVALUATION OF CHEMICALS FOR PECTIN EXTRACTION FROM GUAVA (*PSIDIUM GUAJAVA* L.) FRUITS*

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Sodium hexametaphosphate (SHMP), ammonium oxalate-oxalic acid (AO) and hydrochloric acid (HCl), when used for pectin extraction from winter guava fruits of cultivar 'Sardar' highly improved the crude pectin yield. Low concentrations resulted in high grade pectin and higher number of jelly unit. At higher concentrations, the quality of pectin in terms of equivalent weight, methoxyl content, anhydrogalacturonic acid content and degree of esterification was adversely affected. The chemical aid AO was most effective, followed by SHMP and HCl.

The pectic substances present in plant tissues cannot be completely extracted by boiling with water. The calcium complexing agents like oxalate, polyphosphate and also acids like hydrochloric, nitric, acetic and tartaric have been widely used for improving the extraction[1,2]. Guava is used for jelly making due to its high pectin content. It has also been reported to yield good commercial grade pectin[3]. The present work was undertaken to evaluate some chemicals for their efficiency in pectin extraction from guava fruits and to find out their effect on pectin quality.

Pectin was extracted from winter season harvested guava fruits of cultivar 'Sardar' during December 1979, at two different stages of maturity, namely, (*i*) mature, unripe with average fruit pressure of more than 8.5 kg/cm^2 and (*ii*) partially ripe with a pressure of 6.410 kg/cm^2 as determined with Magness Taylor type pressure tester. The chemicals used for pectin extraction were: (*i*) sodium hexametaphosphate (SHMP) at 0.25, 0.5 and 0.75 per cent concentration; (*ii*) ammonium oxalate and oxalic acid (AO) in 1:1 ratio at 0.25, 0.5 and 0.75 per cent concentration; (*iii*) hydrochloric acid (HCl) at 0.025 N, 0.05 N and 0.075 N concentration and (*iv*) control: only water. The treatments were in triplicates.

Tne pH of extraction media was regulated at 4.0 in case of SHMP, AO and water extraction, by either citric acid or sodium hydroxide solutions. In HCl extraction no pH regulation was done.

Extraction of pectin: Five hundred grams of pulp from freshly harvested guava fruits, was heated in one litre of aqueous solution of different chemicals by boiling for 35 min. The extract was filtered, cooled and pectin was precipitated with iso-propanol containing 0.01 N, HCl at 70 per cent concentration, followed by repeated washings with 95 per cent iso-propanol as described by McCready[1]. The precipitates were dried at 35°C, weighed for crude pectin yield and then ground to pass through 60 mesh screen for further analyses.

Characterization of pectin: The moisture and ash contents of extracted pectins were estimated by the methods described by Ranganna[4]. The equivalent weight, methoxyl content and anhydrogalacturonic acid content were determined by the methods given by Owens *et al*[5]. The degree of esterification was calculated on the basis of methoxyl and anhydrogalacturonic acid contents[6]. The jelly grade of the pectin was determined by relative viscosity method[7] and the jelly units were calculated by multiplying the jelly grade with crude pectin yield obtained from 500 g of guava fruits.

TABLE 1. EFFECT OF CHEMICAL EXTRACTANTS ON YIELD, MOISTURE AND ASH CONTENT OF CRUDE PECTIN FROM GUAVA FRUITS

Concn of extractant	Yield (%)		Moisture (%)		Ash (%)	
	Mature unripe	Partly ripe	Mature unripe	Partly ripe	Mature unripe	Partly ripe
Sodium hexametaphosphate						
0.25%	1.91	1.97	13.4	14.0	1.4	1.6
0.50%	2.77	2.58	11.9	11.8	1.6	1.9
0.75%	2.81	2.77	12.8	12.4	1.8	2.4
Ammonium oxalate—Oxalic acid						
0.25%	2.41	2.20	10.5	12.8	2.4	2.8
0.50%	2.99	2.55	10.7	10.4	2.6	2.7
0.75%	3.13	2.43	12.7	10.3	2.1	2.8
Hydrochloric acid						
0.025 N	2.10	1.95	11.7	10.0	1.6	2.4
0.05 N	2.33	2.21	9.5	10.0	1.6	2.6
0.075 N	2.04	1.71	10.9	11.7	1.7	1.2
Water						
	0.90	1.05	10.2	11.0	1.5	1.8

*Paper presented at the First AFST(I) International Food Conference, 1982, at Bangalore.

TABLE 2. EFFECT OF CHEMICAL EXTRACTANTS ON CHEMICAL QUALITY OF GUAVA PECTIN*

Concn of extractant	Equivalent wt.		Methoxyl content (%)		Anhydrogalacturonic acid (%)		Degree of esterification	
	Mature unripe	Partly ripe	Mature unripe	Partly ripe	Mature unripe	Partly ripe	Mature unripe	Partly ripe
Sodium hexametaphospate								
0.25%	731.3	707.6	5.95	5.27	61.11	57.96	55.29	51.57
0.5%	711.2	641.3	5.09	5.20	56.63	60.02	50.95	49.19
0.75%	584.0	545.9	3.86	4.41	55.24	60.39	39.66	41.43
Ammonium oxalate—Oxalic acid								
0.25%	932.8	855.7	6.13	5.74	57.66	56.59	60.32	57.64
0.5%	779.4	805.8	4.94	4.89	53.61	53.61	52.33	51.76
0.75%	604.1	594.3	4.40	4.35	56.24	58.33	44.34	42.38
Hydrochloric acid								
0.025 N	771.5	789.5	5.00	5.39	54.23	56.00	52.26	54.69
0.05 N	731.8	597.7	4.05	4.69	50.10	59.00	45.84	45.11
0.075 N	633.8	514.6	4.06	4.36	53.97	62.06	42.72	39.79
Water								
	756.4	767.4	5.70	5.43	58.67	56.97	55.10	54.17

*All values are on moisture and ash free basis.

Extraction with chemicals solutions resulted in higher yield of pectin (Table 1). SHMP and AO, due to their calcium complexing property of converting insoluble calcium pectate and protopectin into soluble sodium pectate and pectinic acid[8] gave relatively higher yields than HCl which could hydrolyse only protopectin and did not affect the solubilisation of pectates. Low pectin yields from half ripe fruits indicated its reduction with advancement of ripening[9–11].

Moisture and ash contents of pectins prepared by different procedures are presented in Table 1. Ash content of pectin extracted with AO and from partially ripe fruits with HCl was generally higher.

Equivalent weights of pectin extracted at lower concentration of the chemicals and that from unripe fruits were generally high. This may be due to the presence of pectates or pectinates in unripe fruits and that of pectic and pectinic acids with free carboxylic groups in partially ripe fruits. Similar trend was observed with regard to the degree of methoxylation (Meo).

Maximum anhydrogalacturonic acid (AUA) was present in the pectin extracted with 0.25 per cent SHMP in the case of unripe fruits and 0.075 N HCl in the case of partially ripe fruits. The stage of fruit ripening did not affect MeO or AUA content but, by and large, unripe fruits yielded pectins with higher DE over half ripe ones, indicating deesterification occuring with advancement of ripening[12].

Although chemical solutions extracted pectins with low jelly grade than that observed with water extraction, the yield was considerably higher, thereby helping to procure more number of jelly units, particularly with SHMP and AO extractions (Table 3). The high concentration of the chemicals decreased the number of jelly units.

TABLE 3. EFFECT OF CHEMICAL EXTRACTANTS ON JELLY GRADE AND NUMBER OF JELLY UNITS OF PECTINS FROM GUAVA FRUITS

Concn of extractant	Jelly grade		Jelly units	
	Mature unripe	Partly ripe	Mature unripe	Partly ripe
Sodium hexametaphosphate				
0.25%	132	123	1273	1352
0.50%	116	118	1752	1703
0.75%	107	119	1609	1580
Ammonium oxalate—Oxalic acid				
0.25%	148	142	1745	1645
0.50%	144	135	1872	1676
0.75%	136	123	1682	1476
Hydrochloric acid				
0.025 N	164	152	1560	1346
0.05 N	142	134	1456	1405
0.075 N	116	120	1105	950
Water				
	157	159	662	780

Results indicate that the chemicals especially AO and

SHMP help in obtaining higher yields of pectin with high jelly grade and higher number of jelly units from winter guava fruits of cv. 'Sardar'.

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ENZYMES RELATED TO GLUCONEOGENESIS AND ORGANIC ACID METABOLISM IN RIPENING FRUITS

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Gluconeogenic enzymes, namely, PEPCK, FDPase and G-6 pase as well as glucokinase increased with ripening of mango indicating active gluconeogenesis. PEPCK activity did not change in oranges. Traces of PEPCK activity was noticed in bananas while in oranges there was no change.

Fruits in general accumulate sugars with a simultaneous decrease in organic acids. Besides the contribution made by starch for sugar accumulation, organic acids might contribute to the sugar content via gluconeogenesis. The role of phosphoenolpyruvate carboxykinase (PEPCK) in gluconeogenesis has been indicated in ripening grapes[1]. FDPase involved in gluconeogenesis has been reported to remain unchanged during ripening of banana[2]. This communication reports some of the enzymes connected with gluconeogenesis and organic acid metabolism in mango (*M. indica*, 'Badami' variety), orange (*C. reticulata*, 'Coorg mandarins') and banana (*M. sapientum*, 'Dwarf Cavendish' variety) at different stages of ripening. Freshly harvested and fully matured fruits were used in the present investigation.

By standard CO_2 evolution method (Pettenkofer's method)[3] the various stages of the fruit were determined.

About 200 g pulp was ground in 50 ml medium containing 0.33 M sucrose, 0.01 M ethylenediamine tetraacetic acid (EDTA), 0.01 M potassium phosphate, 0.05 M Tris (pH 8.5), 0.50 per cent polyvinylpyrrolidone (PVP), 0.50 per cent egg albumin and 0.05 per cent cysteine. pH of the medium was adjusted to 7.0 during grinding by the addition of 3M Tris in 0.33 M sucrose. The mitochondrial fraction was sedimented and washed thrice with 0.33 M sucrose. The microsomal fraction was sedimented by centrifuging at 100,000 $\times$ G for 60 min, washed with 0.33 M sucrose and resedimented. The supernatant was dialysed overnight against 0.02 M Tris-HCl buffer (pH 7.2) containing 2 mM EDTA. In the case of raw fruits, dialysis was carried out after precipitation of the supernatant with ammonium sulphate (80 per cent). The dialysed samples were used for enzyme analysis. Phosphoenolpyruvate carboxykinase (PEPCK)[5], phosphoenolpyruvate carboxylase (PEPC)[6], malic enzyme[7] and glucokinase[8] activities in the supernatant fraction were measured spectrophotometrically. Fructose-1,6-diphosphatase[9] activity in the supernatant and microsomal glucose-6-phosphatase[10] activity were determined by estimating the liberated phosphate[11] after the hydrolysis of the respective substrates. The mitochondrial succinate dehydrogenase[12] activity was assayed by following the reduction of 2,6-dichlorophenol indophenol in the presence of phenazinemethosulphate and protein was estimated by the method of Lowry *et al.*[13]. The specific activities of the enzymes were expressed as μ moles of substrates reacted / min / mg protein. Succinate dehydrogenase activity was expressed as change in absorbance at 600 nm/min/mg protein.

As seen in Table 1, activities of gluconeogenic enzymes viz., PEPCK, FDPase and G-6-Pase as well as glucokinase registered a significant increase in mango as the fruit passed through climacteric and reached edible, ripe stage. PEPCK which is involved in the conversion of oxaloacetate into PEP recorded maximum increase in the activity (60 fold) during ripening of mango. In-

TABLE 1. CHANGES IN ENZYME ACTIVITIES DURING RIPENING OF MANGO, BANANA AND ORANGE.

Fruit	Enzyme	Pre-climacteric	Climacteric (sp. activity units)	Post climacteric
Mango	PEPCK	1.0±0.13	26.0±2.8	61.0± 2.8
	FDPase	33.0±2. 1	58.0±2.3	454.0±22.3
	G-6-Pase	23.0±0. 5	—	65.0± 5.4
	PEPC	0.6±0.04	1.6±0.21	25.0± 3.4
	Malic enzyme	38.0±0.11	—	36.0± 0.6
Banana	PEPCK	Traces	Traces	Traces
	PEPC	88.0±0.5	94.0±1.2	92.0± 0.7
	Malic enzyme	122.0±1.7	—	99.0± 6.0
Orange*	PEPCK	30.0±3.2	21.0±2.4	27.0± 1.8
	Malic enzyme	140.0±8.7	128.0±4.5	201.0±26.6

The values are averages of 4 individual fruits ± S.E.M.

*In orange preclimacteric, climacteric and post climacteric correspond to raw green, turning ripe or turning yellow and ripe (yellow) stages of maturity.

creased PEPCK, FDPase and G-6-Pase activities in mango, during ripening indicate the functioning of active gluconeogenesis. Citrate lyase which is known to increase during ripening of mango[14] might supply oxaloacetate (OAA) for PEPCK reaction thus promoting gluconeogenesis. PEPCK activity did not change in oranges.

Among the enzymes related to organic acid metabolism, PEPC which is involved in the synthesis of OAA and hence malic acid, increased (40 fold) in ripening mango during post-climacteric period. The other enzymes which can lead to malic acid synthesis are PEPCK, malic enzyme and malate synthetase. PEPCK functions mostly in the formation of PEP from OAA and malic enzyme in the direction of malic acid decarboxylation. Further, malate synthetase activity has been reported to decrease during the ripening of mango[15]. This emphasizes the possible role played by PEPC in the formation of malic acid in ripening mangoes. Suggestion to this effect has been made in the studies related to organic acid metabolism in grapes[16]. Similarly, PEPC activity which is considerable in banana might play a role in the accumulation of malic acid during ripening of banana. Also malate synthetase activity in banana is known to decrease during ripening[2].

Changes in PEPCK and PEPC levels in banana during climacteric have been reported in literature[17]. In the present study similar observations were made in the case of PEPCK while PEPC activity was detected in traces throughout the period.

In addition to this, previous work[18] has indicated that when[14] C malic acid was injected in mango fruit, 16 per cent of the incorporated label was in sugar fraction in raw fruit, which increased to 26 per cent at ripe stage. In the case of banana however, the incorporation was one per cent both in the raw as well as the ripe stage, indicating lower conversion of malic acid to sugars.

Results published in the above paper have demonstrated that malate[14] C incorporation into sugar in orange fruit segments is of the order of 16 per cent at all stages which is reflected in enzyme levels presented in Table 1.

The present data indicate that gluconeogenesis in mango and accumulation of malic acid in mango and banana might be controlled by the variations in the levels of related enzymes.

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STUDIES ON THE MICROBIOLOGICAL QUALITY OF FISHES REARED IN BIO-GAS EFFLUENTS

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Studies on the microbiological quality and possible transmission of enteric pathogens to humans by fresh water fishes cultured using bio-gas effluents was taken up. It was found that there is less recovery of these pathogens from the fishes grown in bio-gas effluents after primary treatment. The fishes grown in the treated bio-gas effluents are microbiologically comparable to those of fresh-water as far as the viable counts of these enteric pathogens are concerned and the fishes serve merely as passive vectors of human pathogens which can be eliminated by depuration and cooking.

In view of the gradual depletion of quality waters, the hike in chemical fertilizers, the dire necessity of having protein-rich food and the large availability of nutrient-rich bio-gas effluents in our country, it becomes essential to advance large-scale rearing of fishes using these effluents which have proved itself as a valuable fertilizer to boosting fish yield. When animal excreta are used to fertilize fish pond, questions of possible spread of pathogenic organisms that could cause serious diseases in man through fishes arise[1]. Among the cold-blooded animals, fresh-water fish may harbour human enteric pathogens when they are exposed to waters receiving fecal matter[2,3]. Nevertheless, the effluents coming from biogas plants contain reduced number of these enteric pathogens owing to anaerobic digestion[4], and serve as excellent source of manure for fish ponds as demonstrated by the increase in natural fish food (phytoplanktons and zooplanktons) and fish yields[5]. However, since the temperature and retention time are different in the bio-gas digestors, a small percentage of these pathogens escape so that digested effluents may contain demonstrable amounts of virus and bacteria[6,7] which is the focal theme of the present study.

Earlier study in this laboratory[8] on the viable counts of enteric bacteria (total coliforms, *Salmonella* and fecal *Streptococci*) in the three ponds used for fish-culture indicated that there is a gradual reduction in their viable counts after anaerobic digestion and primary treatment. The present study is a follow-up of the previous one to analyse various tissues of these fishes grown in these ponds for the possible presence of these pathogens on par with the fresh water fishes and dam fishes which have not been fertilized with cow-dung slurry.

Pisciculture with fresh-water fishes namely, *Channa striatus* (snake-head), *Saccobrancus* (cat-fish) and *Sorothorodon* (Tilapia) using the digested slurry from gobar-gas (cow dung) plants is going on for the past three years at our college. The digested slurry is being directly fed into the Pond I, namely, the oxidation pond. After the preliminary treatment, i.e., allowing sedimentation for 15 days, the supernatant is regulated to the Pond II, namely, treated water pond where it is further diluted with water. The Pond III, namely, the fresh-water pond which is not fertilized by cow-dung slurry, serves as the control and the fishes are being reared in all the three ponds. The estimation of pH, salinity, total alkalinity, BOD and DO were carried out according to the methods discribed in APHA[9]. Microbiological analyses were carried out during two consequetive years in the same season and the average is given. Fresh tissues of these fishes (muscle and gut region) were screened for the presence of enteropathogens, namely, coliforms, *Salmonella* and fecal *Streptococci* following the methods described in APHA[9] and Geldreich[10]. Surface contamination of the fishes, if any, was eliminated by immersing the fishes in 0.05 per cent mercuric chloride in 50 per cent ethanol for 5 min and then repeatedly rinsing with

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sterile water or saline. The fishes were randomly collected and five fish samples were pooled and ground in pre-enrichment media namely, peptone-water and suitable aliquots of each sample were directly plated into the respective medium. Thus *Salmonella* were enumerated by using the enrichment medium *Salmonella-Shigella* agar, total coliforms by MacConkey agar and fecal *Streptococci* by KF—*Streptococcus* agar following the incubation at 37°C for 24 and 48 hr. Representative colonies were sub-cultured on slants and further characterized for morphological and bio-chemical identification. Isolates were classified according to the schemata of Bailey and Scott[11] and Buchanan and Gibbons[12].

Organic wastes produced by the normal metabolism of animals provide a good source of food for zooplanktons and phytoplanktons[13]. It is evident from the above study that there is a gradual reduction in the number of enteric organisms (Table 1) and the number equals those found in fresh water pond after the slurry has been treated for a number of days (49×10^2 in the treated water pond) as against the highest number in the fresh digested slurry (99×10^2). The pH reported in Pond I, Pond II and Pond III are 9.0, 8.5 and 8.07 respectively. The dissolved oxygen also increased considerably from 4.2 to 6.2 mg/l. The high pH and dissolved oxygen prevailing in these ponds increase the rate of disinfection of coliforms[14] that leads to the reduction of their viable counts.

The harbouring capacity of each fish to different pathogens also varies even when they are grown in the same pond. Thus *Channa striatus* harbours maximum number of *Salmonella* (8.9×10^2/g dry weight) and fecal *Streptococci* (6.1×10^3/g dry weight) and *Saccobrancus* bears maximum number of coliforms (4.2×10^3 to 7.9×10^3). The percentage reduction of these pathogens after primary treatment is reported to be about 32 per cent of *Salmonella*, 62 per cent of total coliforms and 71 per cent of fecal *Streptococci*.

In the present study, highest number of these enteric organisms were recovered from the gut contents of these fishes (Table 2, 3, and 4) than that from the muscle. All available evidences show that fish do not suffer from infections of *Salmonella*, *Shigella* and other enterobac-

Table 1. Enteric pathogens (no. of colonies per ml) from slurry and ponds

	Slurry	Pond I	Pond II	Pond III
Total coliforms	99×10^2	66×10^2	49×10^2	31×10^2
Salmonella	169	117	87	65
Fecal *Streptococci*	507	212	65	26

Table 2. Enumeration of enteric pathogens from the fish *Sorothorodon*

Name of the pathogen	Source of cultivation			
	Pond I	Pond II	Pond III	Dam
Salmonella				
Muscle	6.2×10^2	3.7×10^2	3.2×10^2	3.8×10^2
Gut	6.9×10^2	4.2×10^2	4.4×10^2	4.0×10^2
Total coliforms				
Muscle	3.2×10^3	1.2×10^3	1.6×10^3	2.2×10^3
Gut	4.1×10^3	2.0×10^3	2.8×10^3	2.2×10^3
Fecal *Streptococci*				
Muscle	5.7×10^2	3.2×10^2	3.0×10^2	3.5×10^2
Gut	6.2×10^2	3.8×10^2	3.6×10^2	3.5×10^2

Pond I : Oxidation pond
Pond II : Treated water pond
Pond III : Fresh water pond

Table 3. Enumeration of enteric pathogens from the fish *Saccobrancus*

Pathogen	Pond I	Pond II	Pond III	Dam
Salmonella				
Muscle	3.7×10^2	1.8×10^2	1.6×10^2	1.7×10^2
Gut	4.7×10^2	3.2×10^2	3.2×10^2	3.4×10^2
Total coliforms				
Muscle	6.5×10^3	4.2×10^3	5.8×10^3	5.0×10^3
Gut	7.9×10^3	4.8×10^3	5.1×10^3	5.6×10^3
Fecal *Streptocccci*				
Muscle	4.8×10^3	3.2×10^3	3.9×10^3	4.2×10^3
Gut	5.9×10^3	3.8×10^3	3.2×10^3	4.5×10^3

Table 4. Enumeration of enteric pathogens from the fish *Channa striatus*

Pathogen	Pond I	Pond II	Pond III	Dam
Salmonella				
Muscle	8.9×10^2	5.7×10^2	5.0×10^2	6.1×10^2
Gut	4.1×10^3	1.2×10^3	6.6×10^2	6.4×10^2
Total coliforms				
Muscle	1.3×10^3	6.1×10^3	3.7×10^3	3.2×10^3
Gut	3.0×10^3	1.2×10^4	2.9×10^3	3.8×10^3
Fecal *Streptococci*				
Muscle	6.1×10^3	3.8×10^3	4.1×10^3	4.2×10^3
Gut	1.3×10^4	4.7×10^3	5.6×10^3	5.8×10^3

teriaceae group of organisms[15], but their importance as vectors of human infectious diseases by enteric pathogens cannot be overlooked[16].

The work carried out in this laboratory[8] and the work of Hobson *et al*[1], presented strong evidences that digested slurry from bio-gas plants contain reduced number of pathogens owing to anaerobic digestion and longer retention time inside the digestors.

There is further reduction in their viable counts after primary treatment such as sedimentation[17] and as a result only reduced number of pathogens as may be present in any fresh water pond are present in the treated water pond[8] (Table 1). The high levels of *chlorella* reported in treated water pond[5] might have resulted in the drastic reduction of fecal *Streptococci*[18]. The microbial flora of fishes directly reflects the microbial conditions of the water from which they are taken[2].

It is quite evident that the fishes of the treated water pond are alike to those reared in fresh water and dam as far as the presence of enteric organisms are concerned. Similar results have also been obtained with drastic reduction in coliforms in fishes cultured in sewage[19]. The presence of fish leads to lower bacterial counts[20] when the ponds are fertilized with manure than ponds that received equal amounts of manure but not stocked with fish. Complete elimination of these pathogens may be possible if the fishes grown in the slurry were flushed out in clean water before they are marketed[18].

In conclusion it is found that the fishes fed with digested effluents of bio-gas plants face the same problem of other fishes in their microbial content which could be possibly eliminated by depuration and by cooking.

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STUDIES ON PREPARATION OF *CHAKKA* FROM COW MILK*

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In the efforts to increase yield of *Chakka* from cow milk addition of 2 per cent skim milk powder and 0.3 per cent sodium alginate produced 33.65 per cent outturn which was nearly 10 per cent more than the yield from normal cow milk [3.5% fat and 8.5% solids-not-fat (SNF)] and slightly more than that from buffalo milk (6% of fat and 9% SNF). Organoleptic qualities of this *Chakka* were on par with those of *Chakka* obtained from untreated cow and buffalo milk. Alongwith addition of skim milk powder, it was necessary to add appropriate level of sodium alginate to avoid excessive loss of total solids with *Chakka* whey.

Chakka is the basic material for preparation of *Shrikhand*. Generally buffalo milk is used for manufacture of *Chakka* which gives higher outturn and receives consumer preference. However, cow milk production in Maharashtra is steadily increasing in the last few years[1]. There are problems of getting the yield and general qualities of the cow milk products to compare favourably with those of buffalo milk products. Hence in anattempt to overcome some of these problems, studies were undertaken to prepare *Chakka* from cow milk using skim milk powder (SMP) and sodium alginate in different proportions.

Composite samples of crossbred cow milk obtained from M.P.A.U., Rahuri and commercial buffalo milk samples were used for this study. Cow milk was standardized to 3.5 per cent fat and 8.5 per cent solids-not-fat (SNF) while buffalo milk samples were standardized to 6 per cent fat and 9 per cent SNF.

Three levels of SMP and sodium alginate were selected for study after assessing organoleptic quality of the *Chakka* prepared in pre-experimental trials with combinations of varying proportion of these ingredients. Skim milk powder manufactured by Mehasana District Cooperate Milk Producers Union Ltd., meeting ISI requirements and sodium alginate manufactured by Alimohammad & Co., Bombay were used. In all, eleven treatments were studied in four replications as indicated below:

Level of skim milk powder	Level of sodium alginate (%)		
	(A_1)	(A_2)	(A_3)
0.0 (S_0)	0.1	0.2	0.3
1.0 (S_1)	0.1	0.2	0.3
2.0 (S_2)	0.1	0.2	0.3

Buffalo milk (BM) and cow milk (CM) without any additves were used as control.

The *Chakka* was prepared by the method given in Dairy Handbook, N.D.R.I., Karnal[2]. Additives, viz., SMP and sodium alginate were mixed dry in proportions as fixed for the different treatments and then added into the milk during heating with constant stirring. Active bulk starter culture of *Streptococcus diacetilactis* (DRC_1) was inoculated into the milk at the level of one per cent. The inoculated milk was incubated at 25°C till it was completely coagulated. The curd was hung in a cloth to drain out whey. Weight of each lot of *Chakka* was recorded immediately after the complete drainage of whey. The *Chakka* samples were then stored in refrigerator until analysis.

Milk samples were analysed by ISI methods for fat[3] and SNF[4]. Titratable acidity of the *Chakka* samples was determined according to method recommended for *dahi* in Manual in Dairy Chemistry, ICAR[5]. Fat content was estimated as per the method described by Chaudhari[6] and total solids contents by gravimetric method[5]. Organoleptic evaluation of the *Chakka* was made by ISI method[7]. Total solids content in *Chakka* whey was determined by ISI method described for determination of TS in milk[8].

Acidity: It was observed (Table 1) that there were non-significant differences in the acidity level in *Chakka* due to different treatments. The acidity ranged from 1.09 (A_3S_0) to 1.39 (BM) per cent. The data, however, suggest that with an increased level of sodium alginate the acidity in the product declined. The results further indicated that the buffalo milk *Chakka* had significantly higher acidity (1.39 per cent) than the cow milk *Chakka* (1.26 per cent). The results of this investigation differ from those reported by Zariwala and Sharma[9] and Earnest *et al.*[10]

Fat: It is seen from the data (Table 1) that addition of sodium alginate did not significantly affect fat content in *Chakka* while there was significant decrease in fat content in treatment S_2 over the treatment S_0.

*Part of M.Sc. (Agri) thesis of Sr. author submitted to M.P.A.U., Rahuri.

TABLE 1. EFFECT OF ADDITION OF SKIM MILK POWDER AND SODIUM ALGINATE TO COW MILK ON CHEMICAL QUALITY AND YIELD OF *CHAKKA*

Skim milk powder (%)	Sodium alginate (%) 0.1 (A_1)	0.2 (A_2)	0.3 (A_3)	Mean	±S.E.	CD at 5%
			Acidity (% lactic)			
0.0 (S_0)	1.21	1.28	1.09	1.19	±0.258A	NS
1.0 (S_1)	1.28	1.15	1.20	1.20	±0.258S	,,
2.0 (S_2)	1.26	1.17	1.19	1.21	±0.0447A×S	,,
Mean	1.25	1.20	1.16			
BM				1.39	±0.0447	0.1285
CM				1.26		
			Fat (%)			
0.0 (S_0)	11.76	10.94	11.13	11.27	±0.4250A	NS
1.0 (S_1)	10.88	10.56	10.44	10.63	±0.4250S	1.2222
2.0 (S_2)	10.01	10.00	9.23	9.75	±0.7362A×S	2.1171
Mean	10.88	10.50	10.27			
BM				17.94	±0.7362	2.1171
CM				11.88		
			Total solids (%)			
0.0 (S_0)	30.50	32.29	31.63	31.47	∓0.4208A	NS
1.0 (S_1)	32.98	33.57	32.87	33.14	±0.4208S	1.2102
2.0 (S_2)	33.38	33.93	33.43	33.58	±0.7289A×S	NS
Mean	32.29	33.26	32.62			
BM				33.37	±0.7289	2.0962
CM				32.05		
			Yield (%)			
0.0 (S_0)	24.25	26.90	28.15	26.43	±0.4750A	1.3660
1.0 (S_1)	26.23	28.30	29.93	28.15	±0.4750S	1.3660
2.0 (S_2)	28.93	32.33	33.65	31.64	±0.8228A×S	NS
Mean	26.47	29.18	30.58			
BM				29.81	±0.8228	2.3664
CM				22.60		

NS: Non-significant
BM: Buffalo milk without additive
CM: Cow milk without additive

which is observed due to increased SNF content in the former with the addition of skim milk powder. The *Chakka* obtained from buffalo milk had significantly higher fat (17.94 per cent) than that obtained from cow milk (11.88 per cent) and cow milk subjected to treatments (range 9.23 to 11.76 per cent). The higher fat percentage in buffalo milk *Chakka* was related to the higher percentage of fat in standardized buffalo milk (6.0 per cent) than in standardized cow milk (3.5 per cent). However, the fat content in buffalo milk *Chakka* was found lower than that reported by Ganguli[11] (22.4 per cent) and was slightly more than that given by Earnest *et al.*[10] (15.10 per cent).

Total solids: Total solids (TS) content in *Chakka* obtained from various treatments (Table 1) ranged from 30.50 to 33.93 per cent. There was no considerable difference in TS content of *Chakka* due to treatment with sodium alginate. However, TS content was significantly increased in *Chakka* obtained by addition of SMP (S_1 and S_2) over that obtained without SMP (S_0)

TS in *Chakka* from all treatments including buffalo and cow milk were on par with each other except treat-

ment A_3S_0 (31.63 per cent) and A_1S_0 (30.50 per cent) which were having lower TS content.

Yield: Yield of *Chakka* from various treatments (Table 1) varied from 24.25 to 33.65 per cent. Among the treatments A_2S_2 and A_3S_2 produced significantly more yields, (32.33 and 33.65 per cent, repectively) over all the other treatments including buffalo milk (29.81 per cent). Outturn of *Chakka* from cow milk (22.60 per cent) was, however, significantly lower than that from buffalo milk. It is evident from these results that *Chakka* yield increased consistently by the use of 2 per cent SMP (S_2) in combination with 0.2 and 0.3 per cent sodium alginate by about 10 per cent over untreated cow milk and nearly 3 per cent over buffalo milk *Chakka*. Similar trend of observation was noticed by Aneja *et al.*[12] wherein they found that raising of SNF to 11 per cent could yield 24 per cent *Chakka*, which is 4 per cent more than the normal.

During the investigation the TS lost in the whey were also determined. It was observed that loss of TS decreased with increased level of sodium alginate. On the other hand, contradictory results were noticed when the level of SMP was increased. Appreciably minimum total solids (4.8 per cent) were found in the whey from buffalo milk *Chakka* followed by whey from cow milk *Chakka* (6.83 per cent). Maximum loss was at S_1 level (8.20 per cent). Thus, loss of solids with whey due to addition of SMP in cow milk intended for *Chakka* preparation could be reduced by addition of sodium alginate in proper proportion.

Organoleptic evaluation: The numerical score given by the panel of judges for individual quality attributes of *Chakka* were computed and results obtained are presented in Table 2. It was observed that within the treatments, there was no significant difference in respect of all the organoleptic attributes studied. However, it was observed, in general, that use of sodium alginate alone at higher proportion (A_3S_0) produced inferior quality product. But its use alongwith skim milk powder improved the same. Further, it was also noticed that the *Chakka* from cow milk scored slightly higher than that from buffalo milk and it was preferred by judges particularly for body-texture (8.88) and flavour attributes (8.76). *Chakka* from cow milk with all the additives was acceptable with no appreciable difference in their organoleptic characteristics from those of the control. The results of this investigation show that addition of skim milk powder and sodium alginate together to cow milk, upto 2 and 0.2 percent respectively, could produce matching yield of *Chakka* to that obtained from buffalo milk without affecting its acceptability.

TABLE 2. EFFECT OF ADDITION OF SKIM MILK POWDER AND SODIUM ALGINATE TO COW MILK ON ORGANOLEPTIC ATTRIBUTES OF *CHAKKA*

Quality attribute	Treatment (Means)							
	A_1	A_2	A_3	S_0	S_1	S_2	BM	CM
Colour and appearance	8.60	8.72	8.39	8.45	8.50	8.76	8.56	8.55
Body and texture	8.55	8.42	8.29	8.40	8.29	8.57	8.75	8.88
Flavour	8.62	8.44	8.24	8.37	8.40	8.52	8.27	8.76

Figures in the table are means allotted out of 10.
C.D. at 5% Non-significant.

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A NOTE ON CARCASS AND MEAT CHARACTERISTICS OF BLACK BENGAL MALE GOATS

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Carcass and meat quality parameters were reported in 'Black Bengal' goats. Protein, fat, moisture, sarcoplasmic protein, myofibrillar protein and non-protein nitrogen content of *Longissimus dorsi* were 19.13, 0.94, 77.45, 5.82, 8.90 and 2.01 per cent respectively. The meat colour evaluated subjectively as light red, red and dark red corresponded to a total pigment concentration of 0.24, 0.33 and 0.45 mg/g showing a highly significant ($P < 0.01$) correlation ($r=0.95$) between visual assessment of meat colour and objective estimation of total pigment.

Goats are the principal meat animals in India accounting for about 40 per cent of the total meat production[1]. 'Black Bengal' is a meat breed of goats. Few workers have studied the dressing characteristics of indigenous goats[2–4]. However, no detailed information is available on carcass and meat characteristics of goats reared under farm conditions. The present study was undertaken to provide some basic information on the edible and inedible offal yields, carcass characteristics and physical and chemical properties of meat from male 'Black Bengal' goats from a known herd.

Ten 'Black Bengal' entires, aged about two and half years reared at IVRI, were used in the study. These were sacrificed in the divisional slaughter house following standard procedures. The yields of edible and inedible offals were recorded. The carcass weight included weight of kidneys also. Carcass length was measured from anterior point of aitchbone to anterior edge of the first rib. Carcass was split into two halves between 12th and 13th ribs and the fore- and hind-quarters were weighed. Colour of the eye muscle at 12th rib was subjectively evaluated as light red, red and dark red and given the score of 1, 2 and 3 respectively. Cut muscle area of *Longissimus dorsi* muscle at 10th rib was recorded by marking its outline on a tracing paper and measuring with a compensatory polar planimeter. *Longissimus dorsi* muscle of either sides were separated and analysed for physical parameters of shear force value, cooking release volume and muscle fibre diameter. The chemical analysis for proximate composition[5], sarcoplasmic and myofibrillar[6] proteins and non-protein nitrogen[7] and total pigment[8] were carried out.

TABLE 1. SLAUGHTER AND CARCASS CHARACTERISTICS OF MALE BLACK BENGAL GOATS

Characteristics	Mean±S.E.
Live wt. (kg)	19.25±1.94
Hot carcass wt. (kg)	8.21±1.08
Dressing %	44.68±1.20
Carcass length (cm)	52.55±1.92
Hind quarters as % of hot carcass	43.93±1.10
Fore quarters as % of hot carcass	55.92±1.43
Loin eye area (sq. cm)	5.89±1.57
Skin (% yield)	11.35±1.45
Head (% yield)	9.37±0.94
Pluck and liver (% yield)	3.92±0.79
Caul fat (% yield)	1.87±0.55
Feet (% yield)	2.20±0.59

Results of slaughter and carcass parameters are given in Table 1. The dressing percentage obtained in this study is similar to values reported by earlier workers[2–4] and ranged from 42.88 to 50.68. It was noticed that

TABLE 2. PHYSICAL AND CHEMICAL CHARACTERISTICS OF *LONGISSIMUS DORSI* MUSCLE OF MALE BLACK BENGAL GOATS

Characteristics	Mean±S.E.
Meat colour*	2.2±0.25
Muscle fibre diam. (μ)	29.94±1.37
Cooking release vol (%) (@ 70°C for 20 min.)	30.09±1.34
Shear force value (kg/1.25 cm core)	5.77±0.59
Moisture (%)	77.45±0.31
Crude protein (%)	19.13±0.29
Crude fat (%)	0.94±0.22
Sarcoplasmic protein (%)	5.82±0.26
Myofibrillar protein (%)	8.90±0.34
Non-protein nitrogen (%)	2.01±0.32
Dark red muscle pigment (mg/g)	0.45±0.04
Red muscle pigment (mg/g)	0.33±0.09
Light red muscle pigment (mg/g)	0.24±0.07

*Subjectively evaluated as light red, red and dark red with a score of 1, 2 and 3 respectively.

there was no significant difference in per cent yield of fore- and hind-quarters, showing that the level of development of meat and bone was similar in both the quarters in all the animals.

The physical and chemical properties of *L. dorsi* muscle are shown in Table 2. It was found that both physical and chemical characteristics did not differ significantly between the animals excepting for meat colour and total pigment. A higher value for moisture and lower values for protein and crude fat were observed in this study[9] compared to values for goat meat, however, the breed and sample source are not mentioned[9] in this study which may account for the differences noticed. The values of sarcoplasmic and myofibrillar proteins obtained in this study are presented in Table 2. The meat colour evluated subjectively as light red, red and dark red showed 0.24, 0.33 and 0.45 mg of total pigment per gram of muscle tissue respectively and showed a highly significant ($P<0.01$) correlation ($r=0.95$) between visual assessment of meat colour and objective estimation of total pigment. The data on shear force value, cooking release volume and muscle protein fractions provide basic information on the functional properties, viz., tenderness, water holding capacity and emulsifying capacity respectively and help in determining the suitability of goat meat for incorporation into meat products.

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DEVELOPMENT OF FOOD SNACKS FROM PORK RIND

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Two food snacks, Fried Pork Rind and Minced Fried Pork Rind from pig skin have been developed. Both have a high protein content and are rated from good to very good for their general appearance, flavour, texture, saltinese and overall acceptability.

Pork rind forms about 2.5 to 3.5 per cent by weight of the pig carcasss. Major use of pig skin, in advanced countries, is for gelatin manufacture. In our country fresh pork is sold with backfat and rind on and thus pork rind is cooked as a part of the meat to be eaten as food. Processed rind may be used in comminuted pork products to a maximum of 2 per cent by mass of the meat.[1] Most of the public sector pork processing plants in India have piggery farms attached to them and at some places, pork rind is used in pig feeding. Thus pork rind does not find much economic utilisation in our country and is generally considered as a waste material. Though there are some reports in the literature on the preparation of pig skin as food snacks[2–7], much of this information is secret under patent rights. Present study was undertaken to develop and evaluate the organoleptic acceptability of some snack foods from pork rind using simple technologies for such products that may be adopted under rural conditions with minimum extra investments.

Pork rind was collected from the pigs slaughtered in the Institute. The rind was cut into uniform pieces and scraped with a knife to remove extra fat and any other adhering matter. Cleaned rind was cooked in a household type of pressure cooker (1.02 kg/cm^2) for 5 min to partially cook the rind. The steamed rind was scraped to remove any left over fat and was sun-dried for one day. It may be noted that the partially cooked rind could be fried directly without sun-drying or drying in a hot air oven to a moisture content of 45-50 per cent. In this process the hot frying oil has to be covered for 1-2 min soon after adding rind to avoid splashing of the oil caused by a higher moisture content of the partially cooked rind.

Partially cooked and sun-dried rind was either cut into strips (5 to 10 mm wide; 40 to 60 mm long) or minced once through 4 mm plate of meat mincer. Common salt at 1 per cent level was sprinkled over strips or mixed with minced rind. Strips or minced

TABLE 1. PROXIMATE COMPOSITION OF PARTIALLY COOKED PORK RIND, FRIED PORK RIND AND MINCED FRIED PORK RIND

Type of pork rind	Moisture (%)	Crude protein (%)	Crude fat (%)
Partially cooked	57.57	34.75	4.60
Fried	2.04	71.52	23.05
Minced & fried	0.20	51.97	44.70

TABLE 2. AVERAGE TASTE PANEL SCORES OF FRIED PORK RIND AND MINCED FRIED PORK RIND

Type of pork rind	Appearance	Flavour	Texture	Saltiness	Overall acceptability
Fried	5.66	6.60	6.20	6.00	6.25
Minced & fried	6.33	6.50	6.33	6.00	6.50

Note: Results are expressed on 7-point Hedonic scale where, 7=excellent and 1=very poor.

pork rind was fried in vegetable oil in two stages. First frying was at medium temperature of 155±5°C for 4-6 min or till the products rose to the surface of the oil. Second stage of frying was at a high temperature of 205± 5°C for 30-90 sec or till maximum expansion of the products was achieved. The fried products were strained off excess fat, packed in polythene bags, heat sealed and stored at room temperature.

Partially cooked rind and the fried pork rind products were analysed[8] for proximate composition (Table 1) and the fried products were subjected to taste panel studies (Table 2).

It can be observed from Table 1 that the protein content of fried pork rind (71.52 per cent) and minced fried pork rind (51.97 per cent) is higher than that of partially cooked pork rind (34.75 per cent). This upgrading of protein content of fried products has come as a result of drastic reduction in the moisture content during frying. An increase in the fat content of these products has taken place because of oil pick up during frying, due to their porous cell structures. Both the products are rated from good to very good with regard to their general appearance, flavour, texture, saltiness and overall acceptability.

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CONTROL OF *CYLAS FORMICARIUS* DURING STORAGE OF SWEET POTATO (*IPOMOEA BATATAS*) TUBERS

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Sweet potato tubers heaped on the floor and covered with red earth and wood ash were free from weevil infestation and drying for two months. Storage in gunny bags even with insecticides was not effective beyond one month.

In India, sweet potato is grown over 2.25 lakh hectares with a total production of 13 lakh tonnes. Storage of sweet potato tubers without deterioration is usually difficult on account of heavy infestation by the weevil *Cylas formicarius* Fab.[1–5] Damage may go upto 60 per cent within one month of storage. The harvested tuber may contain the life stages of the weevil which continue the damage during storage. Adults are also attracted to the tuber when kept exposed in the godown. On the tubers the weevils make holes for feeding and egg laying. Grubs bore into the tuber, make tunnels and feed on the internal tissue. The weevil infested tuber is unfit for consumption due to the development of a bitter taste which is attributed to the larva inducing the production of furanoterpene in the tubers[6]. Feeding of tuber by the adult leads to dehydration and shrinking of the tubers and high phenolic values[7].

The usual practice of storage is to keep the harvested tubers exposed in the godown. This leads to heavy

damage due to infestation by the weevil and drying of the tubers. Control of the weevil is very often difficult as the life stages (egg, larva and pupa) are inside the tuber. Earlier workers have reported that fumigating the tubers and treating the bags containing tubers with 5 per cent DDT could reduce weevil infestation; storage of tubers in sand also prevented infestation[8]. The literature regarding the control of weevil during storage is scanty. Studies were hence undertaken to examine the use of various materials in preventing the weevil from gaining access to the stored tubers, the results of which are presented here under.

Sweet potato tubers (variety 'Kanjhangad local') free from weevil infestation were selected immediately after harvest and used for the study. Tubers were dried in sun for 6 hr before storage. Two approaches were adopted in these studies. In one lot, 5 kg each of the tubers were heaped on the floor in a godown and completely covered (5 cm depth) with materials including dried red earth, sand, wood ash and saw-dust and mixtures of sand and red earth with dusts of malathion (5 per cent) and carbaryl (5 per cent). In the other series the tubers were put in small gunny bags and stored in the same godown with or without application of insecticide sprays. The tubers were bagged and insecticide sprays were applied on both surfaces of the bag. In control, tuber heap was left exposed. The temperature and R.H. in the godown were 29 ± 2°C and 70 ± 10 per cent respectively, during the study. Tubers heavily infested with different stages of the weevil were kept in the experiment room to serve as sources of infestation. Four replicates were kept for each treatment. Observations on two replicates were taken after one month of storage and on two sets two months later. Effect of the treatments was assessed based on percentage of tubers infested at the time of observation.

Results presented in Table 1 show that considering the over all effect during the two months of storage, red earth, sand, wood ash and saw dust used alone and mixtures of sand and red earth with dusts of the insecticides, malathion and carbaryl were effective in preventing weevil infestation of the sotred tubers for the entire period. But the tubers under sand and saw-dust treatments dried up when kept for two months, probably because saw-dust and sand could not prevent dehydration of the stored tubers beyond one month. Storage in gunny bags with insecticide treatments was effective in controlling the weevil for one month. After one month, the tuber damage was significant due to weevil infestation as well as drying. Residual toxicity of the insecticides have been lost after one month. In control, hundred per cent tubers were damaged after one month of storage, and when kept for two months they got dried also.

TABLE 1. CONTROL OF *CYLAS FORMICARIUS* DURING STORAGE OF SWEET POTATO TUBERS

Treatments	% of weevil damaged tubers		% of tuber* damage due to weevil + drying
	1 month	2 months	
Tubers covered with dried red earth (20 kg)	0	0	0
Tubers covered with sand (20 kg)	0	0	30.0
Tubers covered with wood ash (5 kg)	0	0	0
Tubers covered with saw-dust (5 kg)	0	0	41.6
Tubers covered with sand (20 kg) + malathion 5% dust (25 g)	0	0	32.3
Tubers covered with red cearth (20 kg) + carbaryl 5% dust (25 g)	0	0	0
Tubers in gunny bag (without spray)	32.1	71.8	83.0
Tubers in gunny bag and surface sprayed with 0.05% fenthion	5.6	38.2	76.2
Tubers in gunny bag and surface sprayed with 0.05% sumithion	5.2	42.4	79.3
Tubers in gunny bag and surface sprayed with 0.05% malathion	6.1	51.2	81.7
Control (Tubers heaped on floor)	100	100	100

*As weevil damage and drying (for 2 months) occur in the same tuber, both the factors are combined.

In red earth and wood ash treatments, tubers remained without any damage upto two months. Both red earth and wood ash when kept as heaps prevented penetration of weevil and prevented dehydration of tubers. Therefore, both red earth and wood ash appeared to be suitable for preserving sweet potato tubers without damage due to the weevil and drying for periods up to two months.

Thanks are due to the Director, Central Tuber Crops Research Institute, Trivandrum, for providing facilities for the above work and to Dr. M. R. G. K. Nair, Retired Professor of Entomology, Kerala Agricultural University, for guidance.

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ERRATUM

In the article entitled "Physico-chemical changes in whole wheat flour (*atta*) and resultant *atta* during storage" by K. Leelavathi, P. Haridas Rao, D. Indrani and S. R. Shurpalekar in JFST Vol. 21, No. 2, 1984, 68–71.

In page 71, the first line in the last para of the text should be read as "It could be concluded that the resultant *atta* has a poor shelf life when compared to *atta* due to higher content of moisture and lipolytic enzymes".

Statement about ownership and other particulars about the periodical entitled JOURNAL OF FOOD SCIENCE AND TECHNOLOGY as required to be published under Rule 8 of the Registration of Newspapers (Central) Rules 1956.

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I, Dr. M. Mahadeviah hereby declare that the particulars given above are true to the best of my knowledge and belief.

M. Mahadeviah
Signature of the Publisher

BOOK REVIEWS

Processing of Fruits, Vegetables and Other Food Products: Published by Small Business Publications, a division of SBP Consultants and Engineers P. Ltd, 4/45, Roop Nagar, Delhi-110007, India; 1983; Price: Rs. 125 (in India), Overseas US $ 35, UK £ 15.

This book deals with different aspects of processing of fruits and vegetables, spices, cereals and plantation produces like coffee, tea, cocoa, cashewnut etc. However, the major emphasis has been on canning and dehydration of fruits and vegetables.

Of the 25 chapters, the first thirteen chapters are devoted to fruit and vegetable products including vinegar production. The first chapter deals with the status and scope of food processing industry in India. Besides h storical development, information on production pattern, product mix, availability of raw materials and other consumables, export potential, problems of the industry and R & D needs have been given.

The next four chapters are devoted to dehydration aspects. Chapter 2 deals with principles of dehydration, its advantages and disadvantages, unit operations involved in dehydration of fruits and vegetables. Chapter 3 deals with different types of driers available for dehydration of food materials in general and fruits and vegetables in particular. A brief discussion on the selection of driers is also included at the end. Chapter 4 is devoted to dehydration of fruits and Chapter 5 to dehydration of vegetables. Method of preparation, recommended temperature and humidity conditions for dehydration of important indigenous fruits and vegetables are discussed.

Canning of fruits and vegetables constitute the subject matter of chapter 6. Various steps involved in canning, principle behind them are briefly covered. Types of cans used, time and temperature of processing for various canned products are tabulated for ready reference. At the end causes of spoilage in canned products and the remedial measures indicated. A section on principle and preparation of intermediate moisture food has found a place here instead under dehydration chapter.

Methods of preparing preserves, candies, glazed and crystalised fruits from different fruits are described in Chapter 7. Similarly, procedures for preparation of jams, jellies and marmalades including some useful recipes are given in Chapter 8. Failures in jam making and corrective measures are indicated at the end.

Chapter 9 relates to the manufacture of traditional products like pickles, chutney and sauces. The preservative principle of these products, method of manufacture are described besides giving some recipes. Causes for spoilage of pickles and sauces indicated.

Chapter 10 deals with fruit juices, beverages, fruit juice concentrates and powders. Steps involved in production of squashes, cordials, nectars and RTS beverages are described in detail and suitable recipes indicated. Various types of evaporators available for concentration of fruit juices and different driers and drying techniques available for preparing fruit juice powders are discussed.

Chapter 11 is devoted to tomato products like tomato juice, tomato puree, tomato paste, tomato ketchup, tomato soup, chilli sauce and tomato chutney, suitable recipe for the above products suggested.

Chapter 12 is exclusively devoted to raw as well as ripe mango products. Mango pickle and chutney, canned slices in syrup, mango beverages like juice, nectar, RTS drinks and squashes, canned pulp, mango cereal flakes and mango custard powder are the important products covered.

Vinegar production both by slow and quick process has been described in chapter 13, besides giving the procedures for production of various types of vinegars.

Convenience foods like dry soups, dry mixes etc. are becoming popular of late in the country. Various formulations and recipes together with method of preparation of such products are described in chapter 14.

Other miscellaneous products like papaya powder, papain and pectin from papaya, papaya fruit slab, papaya candy, pectin from citrus fruits, desiccated coconut, edible products from baell fruit, tamarind juice concentrate and liquid sugar have been dealt in Chapter 15.

Processing of important spices like pepper, cardamom, ginger, chillies, turmeric, cloves, cinnamon, saffron etc. and method of manufacture of their essential oils and oleoresins have been described in Chapter 16.

Chapter 17 relates to cereal products and preparations, malt and malt extracts, protein enriched cereal products, soyabean products, cereal flakes, protein isolates, custard powder formulations and puffed cereals. Methods of their preparation described and their uses indicated.

The next four chapters are devoted to processing of plantation products like cashewnut, cocoa, coffee and tea. Processing of cashew nut and by products of cashewnut industry are covered in chapter 18. Similarly, steps involved in processing of cocoa fruits, cocoa beans, cocoa nibs, cocoa powder and butter, chacolate products and beverages have been discussed in chapter 19. Method of processing coffee fruits, coffee beans, manu-

facture of instant coffee, chicory and decaffeinated coffee briefly described in chapter 20. Methods of manufacture of black tea, instant tea have been included in Chapter 21.

Chapter 22 deals with various steps involved in the production of egg powder including the important step of glucose removal and methods of drying.

Food additives and preservatives have been briefly reviewed and presented in Chapter 23.

Chapter 24 is devoted to food packaging materials like OTS can, glass containers, flexible packaging materials, paper based packaging materials, laminates, cartons, drums etc. Information on filling machines, sealing equipments, labelling machines etc. have been included.

The last chapter deals with waste utilization, by-product recovery, waste and effluent disposal aspects mainly of fruit and vegetable and plantation produce processing industries.

For ready reference, list of ISI specifications available on food products, addresses of manufacturers of additives and preservatives, and of major food processing and packaging machineries in India and suppliers of such equipments in U.S.A. are included in the form of appendix at the end.

A few spelling and printing mistakes have crept into the book. The authors could have given references at the end of each chapter. However, the book is a good source of information on processing of many food products. It will be of use to the general readers and processors in acquainting themselves with the latest processes.

A. M. NANJUNDASWAMY
C.F.T.R.I., MYSORE-13

Spices and Herbs for the Food Industry: by Y. S. Lewis, Food Trade Press, England, 1984; pp. 224, price: £ 26.50.

This book consists of five parts, with a total of 224 pages. The first part deals with the general aspects on (*i*) spices and herbs for the food industry, (*ii*) spice oils, (*iii*) spice oleoresins and (*iv*) methods of test for spices and spice products. In this part, statistical information is given on world production and wholesale price of spices, chief countries producing spices and import of spices into selected countries. Flavour effects of about 40 spices are presented in a tabular form. The preparation of spice oils and oleoresins are also described. The method of testing spices and spice products are presented in a brief and effective manner. The details of the procedures are not given, but references to books giving details are provided.

The second part is devoted to major spices like capsicum, cardamom, ginger, pepper and turmeric. Under each spice, the author has covered agricultural aspects, primary processing, composition of the spice and its products, quality evaluation and uses.

Part three is concerned with tree spices like cinnamon and cassia, clove, nutmeg and mace, pimenta and star anise. Aspects about the plant, harvesting, processing, composition of the spice and its products and their application are also covered.

In part four, minor spices like aniseed, caraway, celery, coriander, cumin, dill, fennel, fenugreek, mustard, poppy and sesame seed, have been covered. Other flavouring commodities like alliums, asafoetida, saffron and vanilla are also included.

The last part deals with leafy spices like basil, bay, marjoram, mint, parsley, sage, thyme, curry leaves etc. Information about the plant, composition and application are given.

This is a good book on spices, covering different aspects, particularly primary processing as well as technological aspects of spices. However the author could have devoted a couple of pages on the utilization of spent meal after extraction.

The printing and the get up of the book are excellent The photographs of some spice plants are impressive and informative. This book should be a valuable addition to libraries of R & D Institutions, Universities and food industries. The students of food science will find it very useful.

N. KRISHNAMURTHY
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Antimicrobials in Foods: Edited by A. L. Branen and P. M. Davidson. Published by Marcel Dekkar Inc. New York and Basel, 1983: pp. 465; Price $ 69.75.

Social and economic changes are taking place with rapid industrialization. There is a shift in the population from the rural to urban areas. This has necessitated conservation and transportation of food from the places of production to those of consumption. In the current marketing system, use of preservatives and antimicrobial agents has become imperative. Eventhough a large number of chemicals are candidates for this purpose, safety of the antimicrobials to humans and animals are of utmost importance. Only a few chemicals belonging to different groups of compounds are legally permitted to be used in foods. Safety of many of the antimicrobials

has been questioned over the past few years and some are totally banned or have limited application. Therefore a thorough knowledge of these permitted antimicrobials is of paramount importance to scinetists and technologists pertaining to the area of food science, including nutritionists, food chemists, toxicologists, microbiologists, biochemists and bacteriologists. The book, "Antimicrobials in foods" definitely provides the necessary information.

In the introductory chapter Dr. Branen discusses the role of additives in food, chemical preservatives, selection of antimicrobials—their antimicrobial spectrum and mode of action, chemical and physical properties, legality and safety of the antimicrobials and prospects of their utility.

The second chapter concerns physical and chemical properties, mechanism of action, antimicrobial activity and applications of sodium benzoate and benzoic acid. Resistance acquired by microorganisms to benzoic acid, its metabolism in microorganisms are also dealt with.

Chemical and physical properties, antimicrobial activity, regulatory status and applications of permitted phenolic compounds, that is, esters of p-hydroxybenzoic acid are presented in chapter three. Naturally occurring phenolic compounds, isolated from different food products, are also discussed in this chapter.

In Chapter IV, organic acids like acetic acid, citric acid, tartaric acid, and some of their salts commonly used in food products have received detailed attention. Next chapter is devoted to medium-chain fatty acids and esters. Sorbates which are commonly used in food products and their characteristics and applications are discussed in the 6th chapter. Sulphur-dioxide and sulfites are the most commonly used preservatives in the food industry. The author of the eighth chapter discusses the source, properties, antimicrobial activity and applications of sulphur dioxide.

Nitrite which is commonly used in meat and in some dairy products is not permitted as a preservative in several countries. The author has reviewed the work so far carried out in this field, which is very informative and could be used as a guide.

In the ninth chapter, chemical sanitizers like halogens, currently used in the food industry are discussed. Information has also been provided on quaternary ammonium compounds, acid-anionic surfatcants and amphotonic surfactants which are used as surface-active agents in food industry. Usefulness of dimethyldicarbonate (DMDC) and diethyldicarbonate (DEDC) which are used for the preservation of wines, fruit juices and soft drinks are discussed in Chapter 10.

Of the several antibiotics tried, only nisin is permitted to be used in the preservation of specific foods in different countries. A few are also used in fruit juices in some countries. Discussion on other inhibitors from lactic bacteria is also briefly given. Antibiotic residues and their significance in food stuffs are discussed by Katz in Chapter 12.

The last chapter is devoted to naturally occurring and miscellaneous food antimicrobials. The products discussed are spices, onion, garlic and vegetable extracts. Antimicrobial substances present in milk and eggs as well as hydrogen peroxide, ethylene diaminetatra acetic acid (EDTA), sodium chloride, phosphates, ethylene oxide, propylene oxide, carbon dioxide, ethanol and propylene glycol also receive brief mention.

Almost all the antimicrobial agents which are now being used in foods as well as those having potential uses have received immense attention. This document is a very useful guide to all persons who have interest in food preservation.

K. R. SREEKANTIAH
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Food Research and Data Analysis: by H. Martens and H. Russwurm Jr. (Eds), Applied Science Publishers, England, 1983; pp. 535; Price: £ 40.00.

This book is the outcome of the proceedings of the second symposium conducted by the International Union of Food Science and Technology in 1982 in Norwegian Food Research Institute, Oslo.

Modern advanced methods of data analysis in food research is emphasised in this book. It has been traditionally applied at different levels in food science and technology and has been stepped up to a greater extent in the recent past. Data on complex product evaluation in product development and quality maintenance, sensory profiling and the many advanced instrumental measures as parameters of quality require multivariate analysis to establish relationship between variables, treatments and groups of samples. Eventhough data analysis is a complicated area, computer technology has revolutionised the same enabling researchers to collect appropriate data, analyse by advanced techniques, interpret and draw valid conclusions creditably for useful scientific achievements.

The book contains 17 full papers and 4 abstracts presented in the symposium. They give details of food products, objectives, data measurements, types of multivariate analysis and interpretation. A very useful subject index classifying them into four broad areas—food application area, sensory measurement methods, chemical and instrumental measurement methods and

data analysis methods is given. The upto date bibliography of references listed yearwise from 1945 is exhaustive and useful.

The editors have included two useful chapters, one on 'Matrix Algebra' which is fundamental to multivariate analysis and the other on 'A Layman's Guide to Multivariate Data Analysis' covering briefly the 9 major groups of multivariate analysis describing different individual models within each group and their areas of application which will be very useful for researchers for a comprehensive understanding of the data measurements, objectives and methods of data analysis. Without these two chapters, occasional exposure to isolated methods and objectives covered in the symposium proceedings will leave the researchers in confusion.

The statistical concepts defined here and there are simple and understandable even to the non-statistician researchers. The two papers on the 'Projections for Computer Hardware Development and Use' and the 'Intelligent Instruments of the Future' give the continued scope and development in the application of data analysis as a useful tool in food research. As the computer network has started to grip all fields in a very advanteous manner, it will be only timely that the food scientists and technologists consider the wider application of multivariate technique for quick and best achievements and this book, no doubt, lays the right emphasis.

S. DHANARAJ
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Physical Properties of Foods: Edited by Micha Peleg and Edward B. Bagley, AVI Publishing Company Inc, West Port, Connecticut 1983; pp. 532; Price $ 45.00.

The book is an outcome of the papers dealing with various aspects of the topic presented at the IFT-IuFoST basic symposium held in 1982 at Las Vegas, Nevada. The important aspects such as (*a*) principles and methods of measuring physical properties including electron microscopy, colorimetry and differential scanning colorimetry, (*b*) structure and other characteristics of plant, animal, synthetic, baked and particulate foods. (*c*) rheology of foods, doughs and emulsions, and (*d*) volatility, expressability, stickiness and phase transitions in low molecular weight carbohydrates have been discussed by seventeen contributers.

Some selected aspects of physical properties of foods such as geometrical, optical, thermal, electrical and mechanical properties have been discussed in the first Chapter. Comprehensive review of principles and techniques of electron microscopy given in the second chapter will be of much use to the scientists and technologists in the field of food research. Theoretical aspects of colorimetry of foods including measurement of colour of foods by spectrophotometers, tristimulus colorimeters and other specialized colorimeters have been discussed briefly in the third Chapter. Denaturation of proteins, gelatinization of starches etc. are some of the physicochemical changes taking place during processing of certain type of foods. The instrumental technique used to study these transition phenomena is referred to as 'Differential Scanning Calorimetry (D.S.C.)'. In the fourth Chapter, the author has reviewed some of the fundamental aspects of D. S. C. and its applications in foods.

Traditionally, emulsions have been explained by the classical two-phase theory considering as two phase system. The intensive research in the field has led to a change of the definition of emulsions. "In an emulsion, liquid droplets and/or liquid crystals are dispersed in a liquid". The conditions necessary for the formation of multilayer emulsions have been discussed in the fifth Chapter.

The relationship between structure and physical properties of meat/muscle tissue, horticultural crops and baked products have been discussed in the sixth, seventh and eighth Chapters respectively, with relevant examples and data including the factors influencing the relation of structure to physical properties.

Chapters nine and ten deal with the physical properties of texturized plant protein materials and measurement and physical characteristics of some food powders (flours, spices, premixed convenience foods etc.).

Emperical tests play a vital role in controlling the process in order to get desired quality in the finished product. A large number of instruments such as penetrometers, compressimeters, viscometers, shearing devices, cutting devices, farinograph, amylograph, Brabender torque rheometer are used to quantify various physical parameters related to maturity, dough rheology and other functional properties. The authors have discussed the response of food systems to applied forces including rheological behaviours of emulsions in Chapters eleven to fourteen using mathematical models.

The flavour and aroma components of some of the foods are being lost during processing, resulting in bland/flavourless finished products. It is possible to achieve good retention of these flavour and aroma components by understanding the mechanism of loss of volatile substances and devising an alternate method of processing. Fifteenth Chapter deals with the physical and

chemical properties governing volatilization of flavour and aroma components. The properties related to "expression" of fluid from different types of foods and prediction of juice yields through mathematical models have been described in Sixteenth Chapter.

The structure of low molecular weight carbohydrates obtained under the water removal process has a significant influence on the properties and quality of food products.

The structure transitions of low molecular weight carbohydrates while converting sugar in solution to the solid state by crystallizstion/drying/freezing methods have been discussed in the Seventeenth Chapter.

The book will be of immense use to scientists, technologists and quality control personnel in the field of food science and technology, and valuable addition to all libraries.

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Grain Quality and Biochemistry: by Umaid Singh, ICRISAT, Patancheru P.O., A.P.—502 324, India, pp: 73.

This is a compilation by Dr. Umaid Singh of the work done at ICRISAT, Hyderabad, on antinutritional factors in chickpea and pigeon pea.

The antinutritional factors studied are trypsin and chymotrypsin inhibitiors, amylase inhibitors, flatulence factors (raffinose, stachyose and verbascose), polyphenols and phytic acids.

The study on protease inhibitors was conducted with 8 *Desi* and 7 *Kabuli* cultivars of chickpea and 3 cultivated and 7 wild species of pigeon pea. The trypsin inhibitor activity (TIA) is present at a higher level in cultivars of chickpea and in the wild species of pigeon pea and TIA is more than chymotrypsin inhibitor activity (CIA). The mature seeds contained more TIA.

Nearly 80-90 per cent of the protease inhibitor activity is destroyed by pressure cooking at 15 lb for 15 min. The open-vessel cooking was less effective in destroying the inhibitor activity. Soaking for 12 hr resulted in only 20 per cent reduction in TIA with little change in CIA.

The *in vitro* protein digestibility (IVPD) was negatively correlated with TIA and CIA, and it varied markedly with cooked samples. The amylase inhibitor activity (AIA) using pancreatic amylase showed large variations among cultivars and was higher in mature seeds.

The polyphenolic compounds were more in dark coloured testa of seeds of both chickpea and pigeon pea, and were more inhibitory than TIA and CIA. Most of the phenolic compounds were located in seed coats and tannins made a very small proportion of the phenolic compounds. Considerable amounts of phenolics were extracted by soaking and boiling and addition of polyvinylpyrrolidone (PVP) markedly reduced the protease inhibitory activity of phenolics.

Chickpea contained more phytic acid as compared to pigeon pea. Cooking markedly reduced the phytic acid in pigeon pea. Germination increased the phytase activity and a concomitant reduction in phytic acid was noted.

The report is a good reference material for workers in the field of antinutritional factors. In addition, it provides a source material for the methodologies employed.

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ASSOCIATION NEWS

Annual General Body Meeting

The Annual General Body Meeting (AGBM) of the Association of Food Scientists and Technologists (India) was held in the afternoon of June 9, 1984 at the Assembly Hall of CFTRI, Mysore. It was attended by 84 members. Lt. Col. O. P. Kapur, the President, was in chair and conducted the proceedings of the meeting.

Dr. S. C. Basappa, the Hon. Executive Secretary, presented the report for 1983–84. In his report, he mentioned that the membership of the Association has increased to 2201, with 12 corporate members and 208 life members. Student membership stood at 219. There are 15 Chapters at present; plans are underway to have a Chapter at Jabalpur and another one in North America for the Indian food scientists settled in U.S.A.

The Awards of the Association for the year 1983 were announced by the Secretary.

Dr. V. Kurien, Chairman, National Dairy Development Board, Anand, was awarded the prestigious Prof. V. Subrahmanyan Industrial Achievement Award.

Laljee Godhoo Smarak Nidhi Award was given to M/s. Larsen and Toubro Limited, R & D Group II, Bombay.

Best Student Award was shared by Mr. Abadan Minoo Kasnavia and Mr. Pradeep Hukimchand Chordia, of Food Technology Training Centre, CFTRI, Mysore.

Gardners Award for the best research paper published in JFST was shared by M/s. Ananthachar, T. K., Narasimha, H. V., Shankara, R., Gopal, M. S. and Desikachar, H. S. R., for their two research papers entitled "Improvement of the Traditional Process for Making Rice Flakes" and "Development of Continuous Process for Making Rice Flakes", published in Vol. 19. No. 2, 1982 and Vol. 19 No. 6, 1982 respectively.

Mr. Abadan Minoo Kasnavia, Training Centre, CFTRI, Mysore was awarded Suman Food Consultants Travel Award for his essay entitled "Role of food science and technology in rural development". A new Award, the Laljee Godhoo Smarak Nidhi Award has been instituted by M/s. Laljee Godhoo and Co., Bombay, from this year. Mr. Ajit Merchant of the company has donated Rs. 10,000 for this Award. The Award is for the highest achievements in R & D in food science and technology.

The report was later adopted and approved by the general body.

Mr. Radhakrishnaiah Setty, the Hon. Treasurer, presented the accounts of the Association for the year and the budget for the next year.

The amendments suggested to the various items of the Bye-Laws of the constitution brought before the meeting were discussed. The General Body gave its approval to enhance the membership fee in view of the increasing cost of production of the JFST.

The names of office bearers, who have got elected for the year 1984–85, were announced by the Hon. Exec. Secretary.

President designate
Dr. A. G. Naik Khurade, Suman Food Consultants, Delhi.

Vice-President (Head Quarters)
Dr. S. S. Arya, DFRL, Mysore.

Vice-Presidents (Chapters)
Dr. Sudhirkumar Gupta, Karnal,
Mr. Y. K. Kapur, Delhi.
Mr. V. C. Sane, Bombay,
Mr. B. Raghuramaiah, Madras.

Hon. Joint Secretary
Dr. D. Narasimha Rao, CFTRI, Mysore

Hon. Treasurer
Dr. Nasirulla, CFTRI, Mysore.

The President, Lt. Col. O. P. Kapur, then inducted the new office bearers, the President, Sri Laljeet Singh, Modern Foods, New Delhi; the Hony. Secretary, Dr. M. Mahadeviah and the other office bearers.

Sri Laljeet Singh, the new President took the chair and outlined his future plans for the Association and requested the help of all members to make his plans fruitful. He also thanked the outgoing Executive Committee members, especially Lt. Col. O. P. Kapur and Dr. S. C. Basappa for running the Association effectively during their tenure of office.

Fourth Indian Convention of Food Scientists and Technologists

The Fourth Indian Convention (ICFOST) was held from June 7-9, 1984, at the Central Food Technological Research Institute, Mysore.

At the inaugural function, the President of AFST (I), Lt. Col. O. P. Kapur, welcomed the delegates and presented the theme of the symposium—Additives in Processed Foods. Dr. B. L. Amla, Director, CFTRI, inaugurated the Symposium. The Keynote address on

'Concepts of Food Safety Evaluation' was delivered by Dr. C.R. Krishnamurthy, Ex-Director, ITRC, Lucknow. The Souvenir was released by Dr. T. R. Sharma, Director, DFRL, Mysore.

There were two special lectures in the morning on 'Applications of plant cell and tissue culture in biotechnology' by Dr. M. Chadha of BARC, Bombay and 'Status of electrolytic tinplate and tin free steel in tinplate industry in India' by Mr. M. C. Kumara Swamy of M/s. Tinplate Co. of India Ltd., Jamshedpur.

The poster sessions were also arranged. They dealt with all aspects of food technology including cereals and pulses, oilseeds, proteins, fruits and vegetables, dairy products, microbiology and fermentation, meat, fish and poultry, quality control and contaminants, infestation control and pesticides, nutrition, food packaging, milling baking and confectionery, food additives, lipids, food engineering and chemistry/bio-chemistry, the abstracts of which have been published in the Souvenir; there were about 250 abstracts presented at the poster sessions.

At the Symposium session on 'Additives in Processed Foods', eleven papers were read. They included place of natural colours in processed foods, role and application of emulsifiers in bakery products, novel sweetners for the food industry, nature and role of acidulants in food processing and modern concept and practice of chemical preservatives, natural and synthetic oxidants, status and role of food colloids, role of sorbic acid in processed foods, role of additives in imparting specific functional properties in processed protein foods, Regulatory aspects on the use of additives in processed foods and regulatory aspects and policy option on food additives in enrichment of foods were read and discussed.

A plenary session was held in the evening and recommendations were drafted.

Industrial Achievement Awards Function

In the afternoon, the awards function was held under the Presidentship of Prof. K. S. Hegde, Vice-Chancellor, Mysore University. Dr. V. Kurien, Chairman, National Dairy Development Board, Anand, was presented the Prof. V. Subrahmanyan Industrial Achievement Award. The award carried a citation, a plaque and cash presentation. After receiving the Award, Dr. Kurien gave a lecture on dairy establishment in Anand, Gujarat.

Dr. V. Kurien receiving Prof. V. Subrahmanyan Industrial Achievement Award from Dr. K. S. Hegde.

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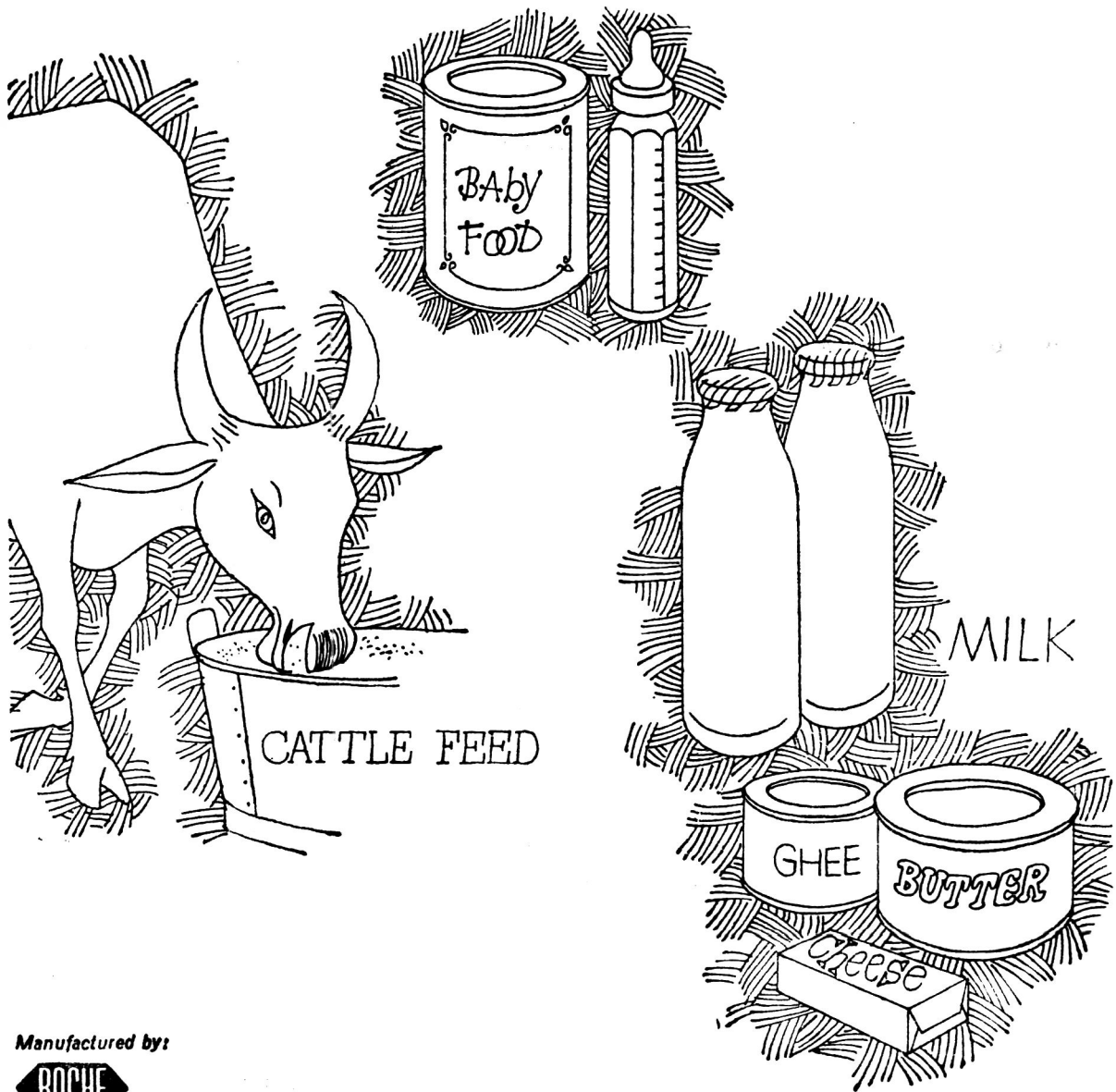

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2. Short communications in the nature of Research Notes should clearly indicate the scope of the investigation and the salient features of the results.

3. Names of chemical compounds and not their formulae should be used in the text. Superscripts and subscripts should be legibly and carefully placed. Foot notes especially for text should be avoided as far as possible.

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

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

6. **Illustrations:** Graphs and other line drawings should be drawn in *Indian ink* on tracing paper or white drawing paper preferably art paper. The lettering should be in double the size of printed letters. For satisfactory reproduction, graphs and line drawings should be at least twice the printed size 16cms (ox axis) × 20cms (oy axis); photographs must be on glossy paper and must have good contrast; *three copies* should be sent.

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

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

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

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

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

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

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

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Research Papers

Research Notes

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